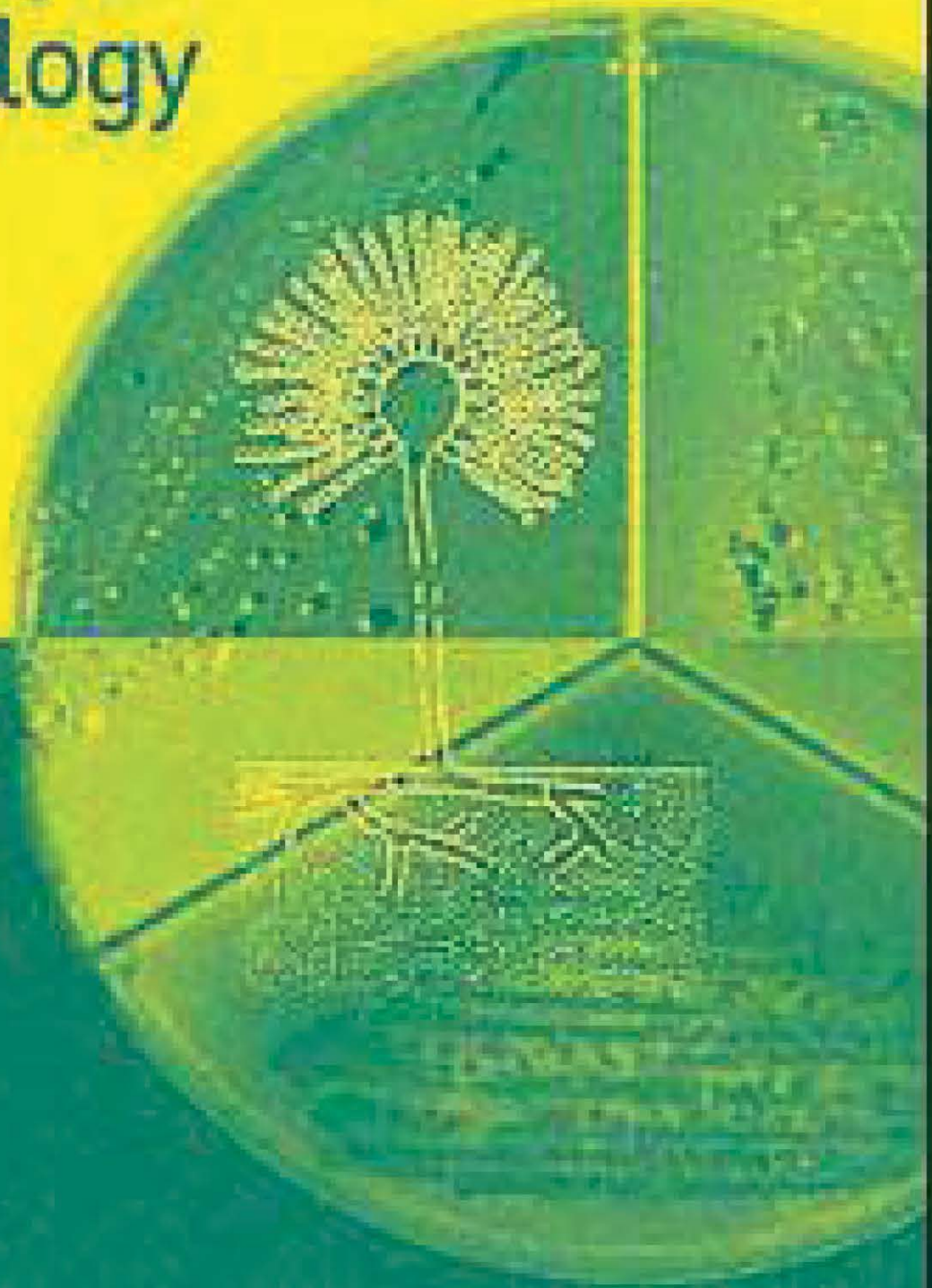
Concise Review of Veterinary Microbiology



P. J. Quinnand
B. K. Markey

Blackwell Publishing

Concise Review of Veterinary Microbiology

P.J. Quinn MVB, PhD, MRCVS
Professor Emeritus
Former Professor of Veterinary Microbiology and Parasitology
Faculty of Veterinary Medicine
University College Dublin

B.K. Markey MVB, PhD, MRCVS, Dip Stat
Senior Lecturer in Veterinary Microbiology
Department of Veterinary Microbiology and Parasitology

Computer graphics by
D. Maguire AIMLS
Department of Veterinary Microbiology and Parasitology

Blackwell Publishing

Editorial Offices:
Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK
Tel: +44 (0)1865 776868
Blackwell Publishing Professional, 2121 State Avenue, Ames, Iowa 50014-8300, USA
Tel: +1 515 292 0140
Blackwell Publishing Asia Pty Ltd, 550 Swanston Street, Carlton, Victoria 3053, Australia
Tel: +61 (0)3 8359 1011

First published 2003 by Blackwell Publishing Ltd

6 2009

Library of Congress Cataloging-in-Publication Data

Quinn, P.J.
Concise review of veterinary microbiology / P.J. Quinn, B.K. Markey ; computer graphics by D. Maguire.
p. ; cm.
Includes index.
ISBN: 978-1-4051-0898-0 (alk. paper)
1. Veterinary microbiology. I. Markey, B.K. (Bryan K.) II. Title.
[DNLM: 1. Animal Diseases—microbiology. 2. Animals—microbiology. 3. Veterinary Medicine. QW 70 Q77c 2003]
SF780.2.Q56 2003
636.089'69041—dc22

2003059381

ISBN: 978-1-4051-0898-0

A catalogue record for this title is available from the British Library

Printed and bound in Singapore
by Utopia Press Pte Ltd

The publisher's policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufactured from pulp processed using acid-free and elementary chlorine-free practices. Furthermore, the publisher ensures that the text paper and cover board used have met acceptable environmental accreditation standards.

For further information on Blackwell Publishing, visit our website:
www.blackwellpublishing.com

Contents

Preface

Although written primarily for veterinary students, this book should be of interest to veterinary graduates who wish to revise veterinary microbiology and microbial diseases. Students and graduates in cognate subject areas may find it useful as an introduction to pathogenic microorganisms, many of public health importance.

The book is divided into three distinct sections: bacteriology, mycology and virology. Introductory chapters in each section explain the terms used, the basis of classification and morphological features of microbial organisms. Individual chapters deal with related pathogenic microorganisms or those with similar morphological and cultural characteristics. In some instances, large families of pathogenic microorganisms have been allocated two chapters in accordance with their importance as agents of infectious disease in animals. For effective utilization of space and for uniformity in layout, two or more unrelated microbial pathogens have been included in one chapter in a few instances. The recommended reclassification of some rickettsiae and the renaming of other rickettsiae as *Mycoplasma* species has been adopted in this book.

Acknowledgements

We wish to acknowledge the assistance we received from colleagues and from family members: Dr. W.J. Donnelly, Lesley Doggett, Professor S. Fanning, Mary Gleeson, Sean Hogan, Barbara Markey, Dr. G. McCarthy, Deirdre Uí Bhrógáin, Michael, John, David and Joan Quinn.

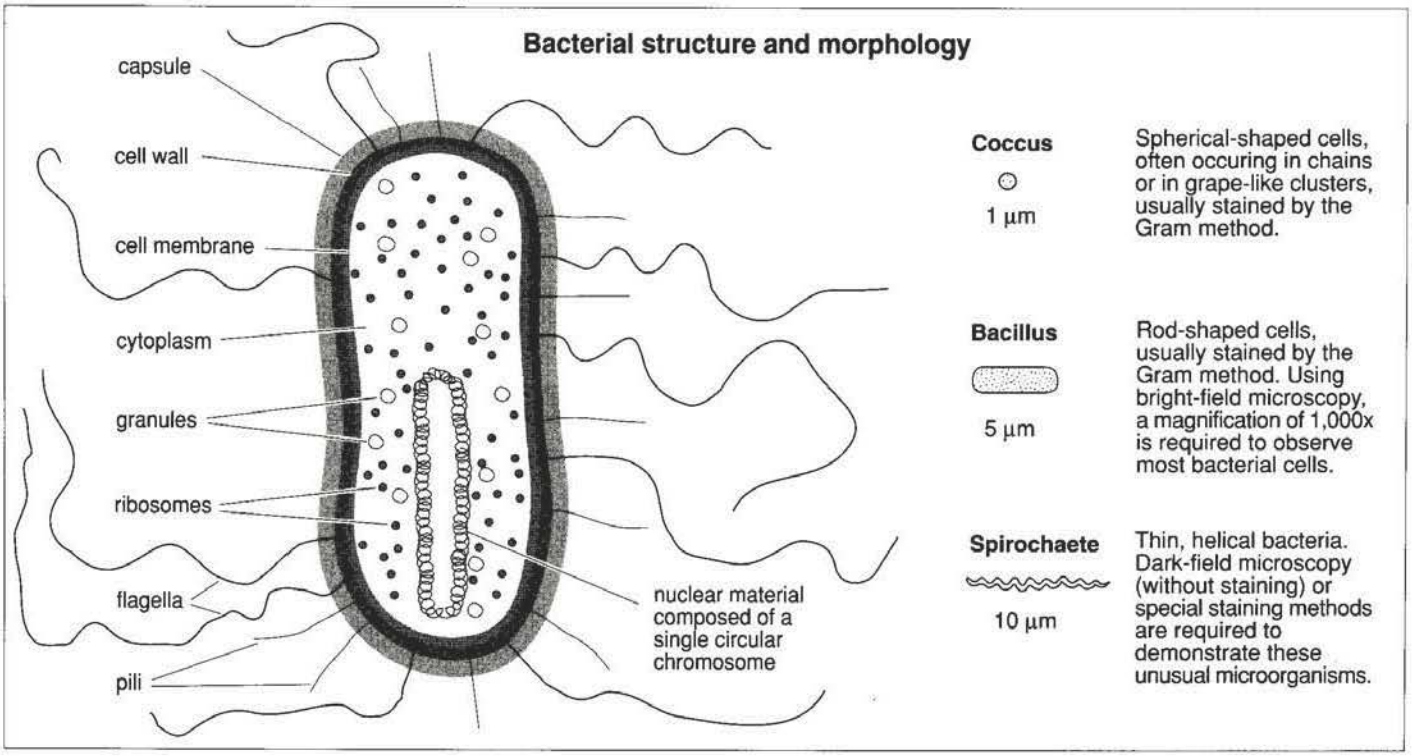
Ms. Antonia Seymour and Ms. Emma Hoyle at Blackwell offered constructive comments on layout and presentation of text, tables and diagrams.

Dublin, June 2003

Section I

Introductory Bacteriology

1 Structure of bacterial cells



Bacteria are unicellular and usually occur in simple shapes such as rods, cocci, spiral forms and occasionally as branching filaments. They usually have rigid cell walls containing a peptidoglycan layer and multiply by binary fission. Bacteria are smaller and less complex than eukaryotic cells and do not contain membrane-bound organelles. Their genetic information is contained in a single circular chromosome; a nuclear membrane and a nucleolus are absent. Despite their morphological diversity, most bacteria are between 0.5 µm and 5 µm in length. Motile bacteria possess flagella by which they can move through liquid media. The majority of bacteria can grow on suitable inert media; some require growth supplements and particular atmospheric conditions for growth.

Most bacteria found in nature are not harmful to humans, animals or plants. Some bacteria make an important contribution to the utilization of nutrients in soil, in water and in the digestive tracts of animals. Bacteria which can cause disease in animals or humans are referred to as pathogenic bacteria.

A typical bacterial cell is composed of a capsule, cell wall, cell membrane, cytoplasm (containing nuclear material) and appendages such as flagella and pili. Some species of bacteria can produce forms termed spores or endospores, structures

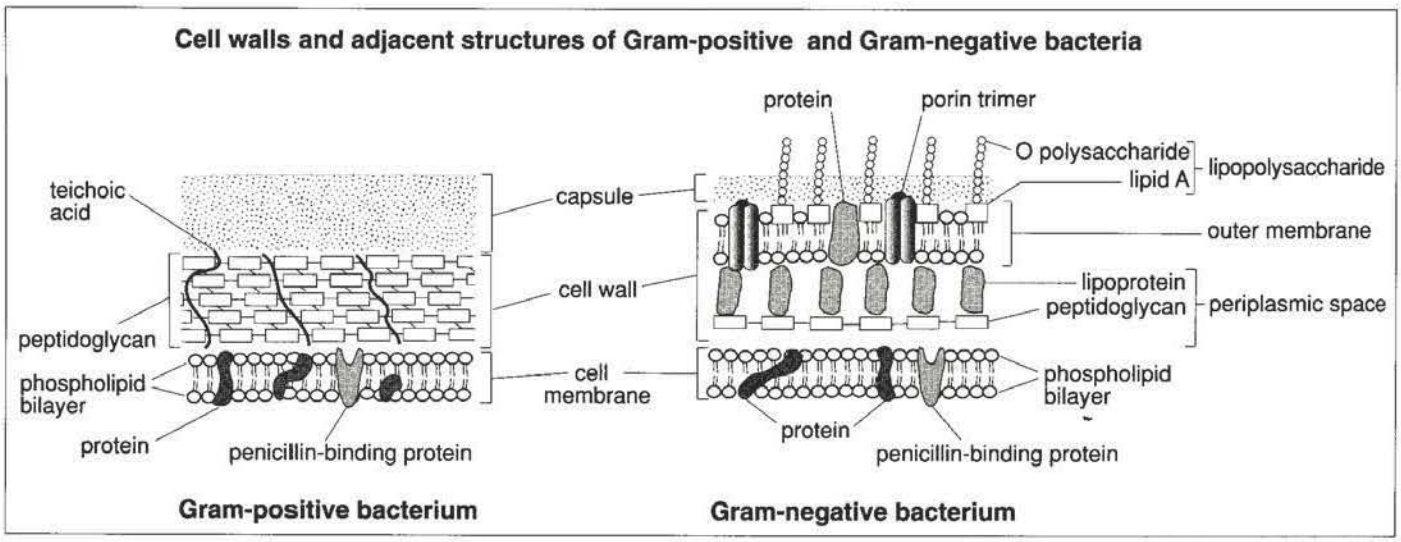


Table 1.1 Structural components of bacterial cells.

Structure	Chemical composition	Comments
Capsule	Usually polysaccharide; polypeptide in *Bacillus anthracis*	Often associated with virulence; interferes with phagocytosis; may prolong survival in the environment
Cell wall	Peptidoglycan and teichoic acid in Gram-positive bacteria. Lipopolysaccharide (LPS), protein, phospholipid and peptidoglycan in Gram-negative bacteria	Peptidoglycan is responsible for the shape of the organism. LPS is responsible for endotoxic effects. Porins, protein structures, regulate the passage of small molecules through the phospholipid layer.
Cell membrane	Phospholipid bilayer	Selectively permeable membrane involved in active transport of nutrients, respiration, excretion and chemoreception.
Flagellum (plural, flagella)	Protein called flagellin	Filamentous structure which confers motility
Pilus (plural, pili)	Protein called pilin	Also known as fimbria (plural, fimbriae). Thin, straight, thread-like structures present on many Gram-negative bacteria. Two types exist, attachment pili and conjugation pili.
Chromosome	DNA	Single circular structure with no nuclear membrane
Ribosome	RNA and protein	Involved in protein synthesis
Storage granules or inclusions	Chemical composition variable	Present in some bacterial cells; may be composed of polyphosphate (volutin or metachromatic granules), poly-beta-hydroxybutyrate (reserve energy source), glycogen

which are resistant to environmental influences. The principal structural components of bacterial cells are presented in Table 1.1. Some bacteria can synthesize extracellular polymeric material, termed a capsule, which forms a well-defined structure, closely adherent to the cell wall. In the body, capsules of pathogenic bacteria interfere with phagocytosis. The tough, rigid cell walls of bacteria protect them from mechanical damage and osmotic lysis. Differences in the structure and chemical composition of the cell walls of bacterial species account for variation in their pathogenicity and influence other characteristics, including staining properties. Mycoplasmas, an important group of bacteria, do not have cell walls.

Bacteria can be divided into two major groups, Gram-positive and Gram-negative, on the basis of colour when stained by the Gram method. This colour reaction is determined by the composition of the cell wall. Gram-positive bacteria, which stain blue, have a relatively thick uniform cell wall which is composed mainly of peptidoglycan and techoic acids. In contrast, Gram-negative bacteria, which stain red, have cell walls with a more complex structure, consisting of an outer membrane and a periplasmic space containing a comparatively small amount of peptidoglycan.

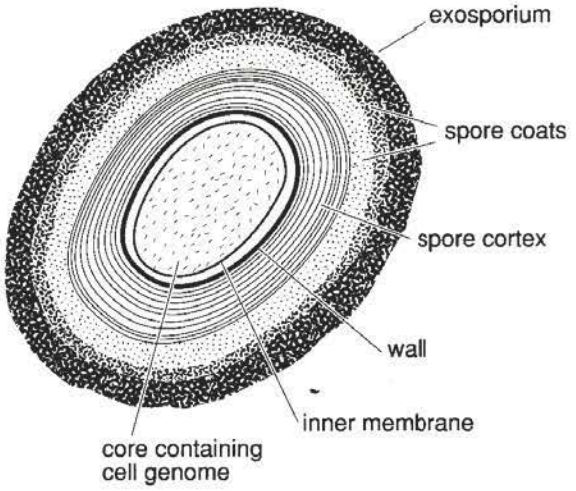
The cell membranes of bacterial cells are flexible structures composed of phospholipids and proteins. Active transport of nutrients into the cell and elimination of waste metabolites are functions of the cell membrane. The cytoplasm, which is enclosed by the cell membrane, is essentially an aqueous fluid containing the nuclear material, ribosomes, nutrients, enzymes and other molecules involved in synthesis, cell maintenance and metabolism.

The bacterial genome is composed of a single haploid circular chromosome containing double-stranded DNA. Bacterial genomes differ in size depending on the species. Plasmids, small circular pieces of DNA which are separate from the genome, are capable of autonomous replication. Plasmid DNA may code for characteristics such as antibiotic resistance and exotoxin production. All protein synthesis takes place on ribosomes, structures composed of ribonucleoproteins.

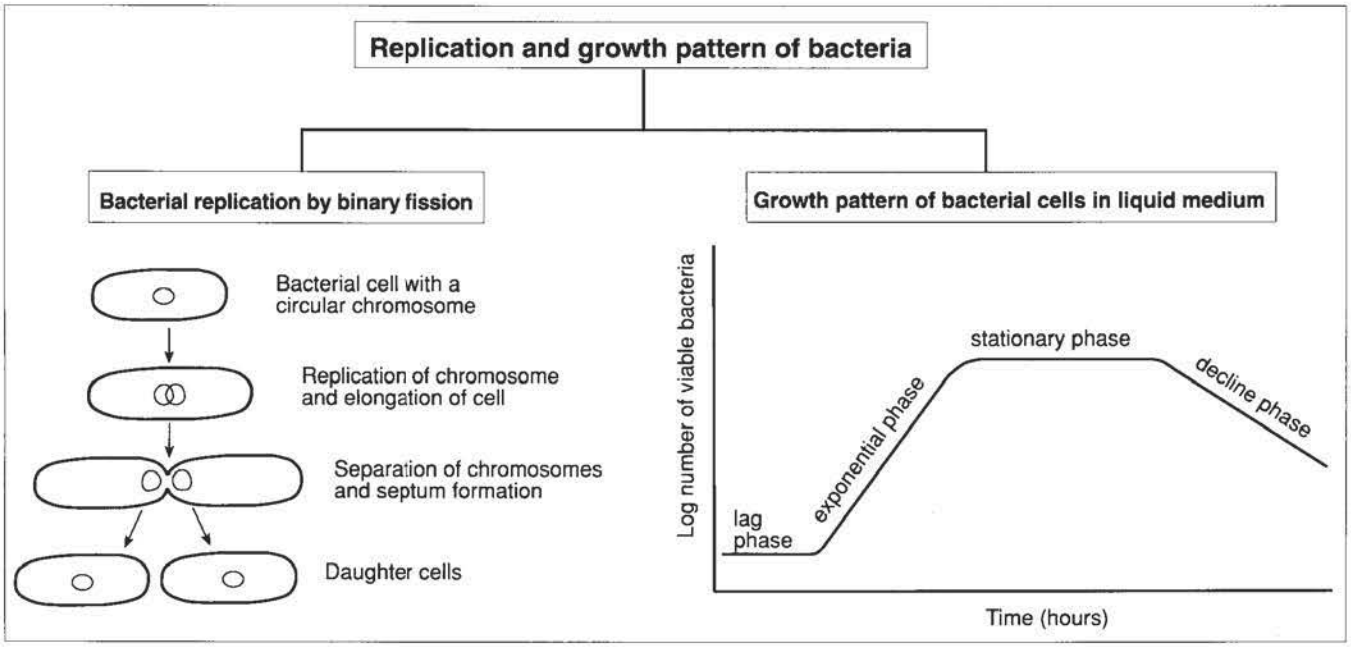
Motile bacteria possess flagella, attached to the cell wall, which are usually several times longer than the bacterial cell and are composed of a protein called flagellin. Fine, straight, hair-like structures called pili or fimbriae, composed of the protein pilin, are attached to the cell wall of many bacteria. In pathogenic bacteria, pili function as adhesins for receptors on mammalian cells.

Dormant, highly resistant bodies, termed endospores, are formed by some bacteria to ensure survival during adverse environmental conditions. The only genera of pathogenic bacteria which contain endospore-forming species are *Bacillus* and *Clostridium*. The resistance of endospores is attributed to their layered structure, their dehydrated state, their negligible metabolic activity and their high content of dipicolinic acid. Because endospores are thermostable, they can be destroyed with certainty only by moist heat at 121°C for 15 minutes.

Structural features of a mature bacterial endospore



2 Cultivation, preservation and inactivation of bacteria



Appropriate conditions of moisture, pH, temperature, osmotic pressure, atmosphere and nutrients are required for bacterial growth. Bacteria replicate by binary fission. The generation time, that is the length of time required for a single bacterial cell to yield two daughter cells, ranges from 30 minutes to 20 hours. Long-term preservation of microorganisms usually involves freezing procedures. Heat treatment or chemicals can be used to inactivate bacteria.

Following inoculation of bacterial cells into fresh broth medium, the growth curve of the culture exhibits lag, exponential and stationary phases and a final decline phase. Binary fission of the young cells results in an exponential increase in numbers. Cell numbers can be determined either as a total cell count or as a viable cell count. Bacteria can be counted by direct microscopy, by colony counting, by membrane filtration and by electronic methods. Accurate cell counts may be required for specific purposes such as vaccine preparation and for bacterial testing of water.

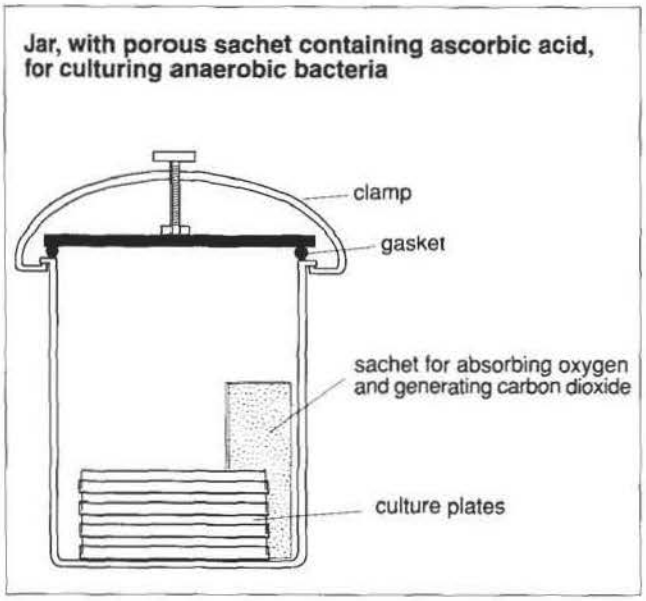
Bacteria acquire nutrients from their immediate environment. Nutrient media for the isolation of pathogenic bacteria are formulated to supply particular growth factors for specific groups of organisms. Most bacteria require carbon and nitrogen in relatively large amounts. Trace elements and certain growth factors such as vitamins are also essential for bacterial growth.

In addition to nutritional factors, growth of bacteria is influenced by genetic factors and by chemical, physical and other environmental influences. Growth of bacteria in culture is influenced by temperature, hydrogen ion concentration, availability of moisture, atmospheric composition and osmotic pressure. Most pathogenic bacteria can be grown aerobically on a nutrient agar medium at 37°C, close to body temperature. Bacteria with an optimal incubation temperature of 37°C are termed mesophiles. Those with an optimal incubation temperature of 15°C are termed psychrophiles and those with an optimal incubation temperature close to 60°C are termed thermophiles. Most bacteria grow optimally at neutral pH.

Based on their preference for particular levels of oxygen, bacteria can be assigned to four main groups, namely aerobes, anaerobes, facultative anaerobes and microaerophiles. A fifth group, capnophiles, are aerobic bacteria with a requirement for carbon dioxide. Anaerobic bacteria are unable to grow in an atmosphere containing oxygen. Strict anaerobes are cultured in tightly sealed jars in an atmosphere from which free oxygen has been removed.

Subculturing can be used for the short-term preservation of bacteria. Limitations of this procedure include death of some cells and a risk of contamination and mutation. Long-term methods of preservation include freeze-drying (lyophilization), freezing at –70°C and ultra-freezing in liquid nitrogen at –190°C. If properly used, these preservation methods can maintain organisms in a hypobiotic state for more than 30 years and ensure that the organisms remain unchanged and uncontaminated.

Sterilization is the method employed for the destruction of microorganisms on equipment used in microbiological and surgical procedures. Physical and chemical methods can be used for inactivation of microorganisms. Chemical agents include antimicrobial drugs, disinfectants and food preserva-



tives. Methods for preventing spoilage or limiting microbial growth in food are presented in Table 2.1. Physical methods for sterilizing equipment or fluids are presented in Table 2.2. Sterilization procedures are effective for the destruction of bacterial, fungal and viral agents. When dealing with bacterial endospores, such as those of *Clostridium* species, heating at a temperature of 121°C for 15 minutes is required for inactivation. Unconventional infectious agents such as prions require more vigorous sterilization procedures. These resistant agents are not inactivated by prolonged heating at 121°C but may be inactivated by heating at 132°C for 4.5 hours.

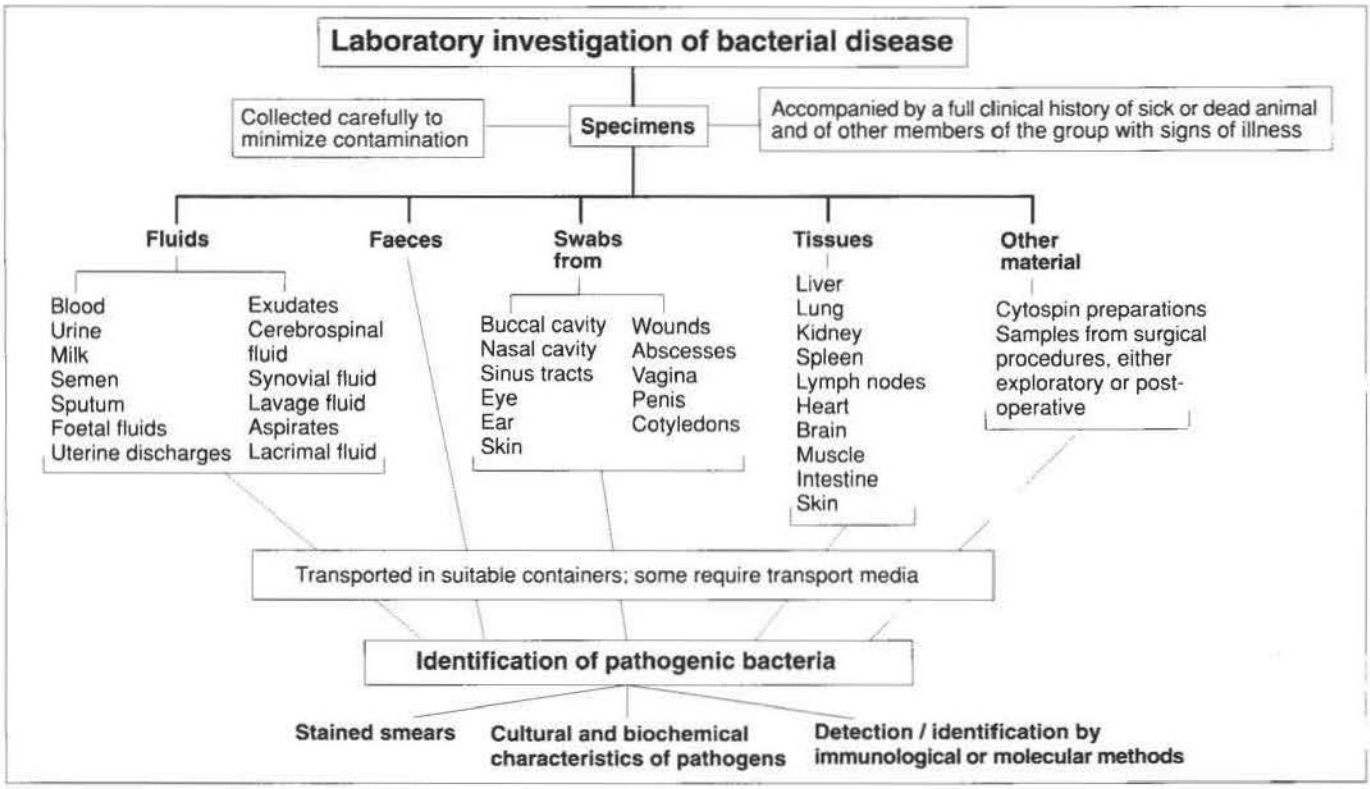
Table 2.2 Physical methods for sterilizing equipment or fluids and for disposing of contaminated material.

Method	Comments
Moist heat (autoclaving) employing steam under pressure to generate 121°C for 15 minutes or 115°C for 45 minutes	Used for sterilizing culture media, laboratory items and surgical equipment. Inappropriate for heat-sensitive plastics or fluids. Prions are not inactivated by this treatment
Dry heat in a hot-air oven at 160°C for 1 to 2 hours	Used for sterilizing metal, glass and other solid materials. Unsuitable for rubber and plastics
Incineration at 1,000°C	Used for destruction of infected carcasses and other contaminated material; environmental pollution a possible outcome
Flaming	Used for sterilizing inoculating loops in the naked flame of a Bunsen burner
Gamma irradiation	Ionizing rays used for sterilizing disposable plastic laboratory and surgical equipment. Unsuitable for glass and metal equipment
UV light	Non-ionizing rays with poor penetration. Used in biosafety cabinets
Membrane filtration	Used for filtering out bacteria from heat-sensitive fluids such as serum and tissue culture media. Pore size of filter should be 0.22 µm or less

Table 2.1 Methods for preventing spoilage and limiting microbial growth in food.

Method	Application	Comments
Refrigeration at 4°C	Prevention of growth of spoilage organisms and pathogenic bacteria	Pathogens such as *Listeria monocytogenes*, *Yersinia* species and many fungal species can grow at 4°C
Freezing at –20°C	Long-term storage of food. Microbial multiplication prevented	Surviving microorganisms can multiply rapidly when thawed food is left at ambient temperatures
Boiling at 100°C	Inactivation of vegetative bacteria and fungi in food	Many endospores can withstand prolonged boiling
Pasteurization at 72°C for 15 seconds	Inactivation of most vegetative bacteria	Heat treatment should be followed by rapid cooling. If present in high numbers, some bacteria may survive
Acidification	Adjustment of pH to a low level inhibits bacterial growth	Applicable to a limited range of foods such as vegetables
Increasing osmotic pressure	Inhibition of microbial multiplication; used for preservation of food	Addition of salts or sugars increases osmotic pressure; applicable to a limited range of foods
Vacuum packing	Packaging of meat and other perishable foods	Removal of oxygen prevents the growth of aerobes
Irradiation	Inactivation of spoilage organisms and pathogenic bacteria	Not permitted in some countries

3 Laboratory diagnosis of bacterial disease



Laboratory investigation of bacterial disease is necessary for the identification of the aetiological agent and sometimes for determining the antimicrobial susceptibility of bacterial pathogens. A full clinical history, including the age and sex of the species affected together with the number of animals involved and any treatment administered, should accompany the specimens. A tentative clinical diagnosis should be included.

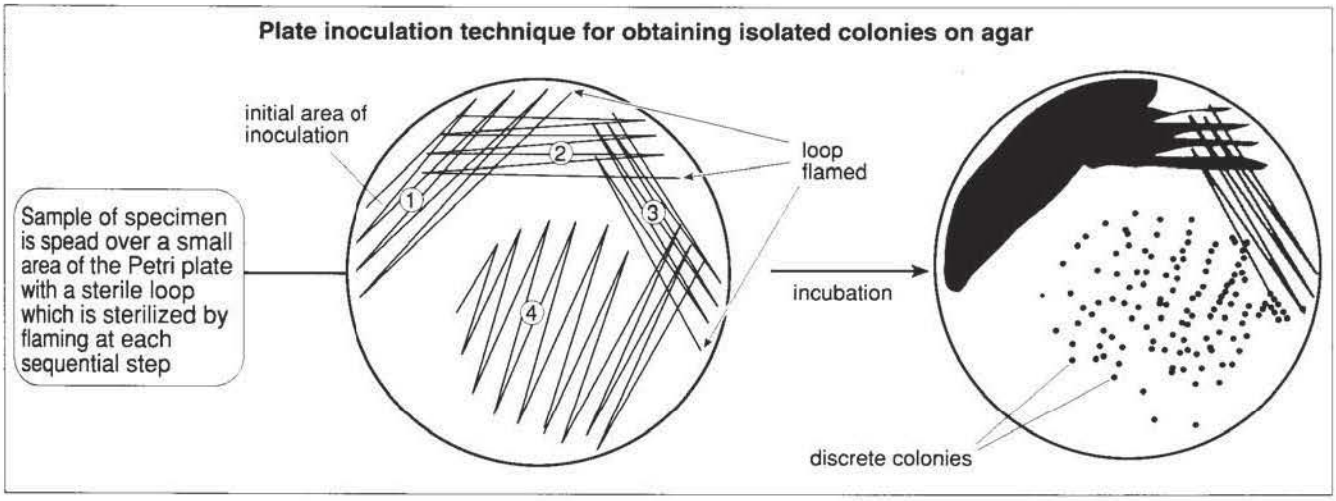
Care should be taken in the selection, collection and submission of specimens to the laboratory. Ideally, specimens should be obtained from live animals before administration of antimicrobial therapy. Samples from dead animals should be collected, if possible, before putrefactive changes occur. Procedures which minimize contamination should be used during specimen collection. Samples must be submitted in separate leak-proof containers. Each container should be labelled with the identity of the animal, the type of specimen and the date of collection.

The presence of pathogenic bacteria can be confirmed by examination of stained smears; cultural and biochemical characteristics and immunological and molecular methods are used for specific identification. Staining methods routinely used in diagnostic bacteriology are presented in Table 3.1. Gram-stained smears from tissues or exudates are useful rapid procedures for demonstrating bacteria present in large numbers. The Ziehl-Neelsen stain is used to detect pathogenic mycobacteria. *Coxiella burnetii*, *Brucella* species, *Nocardia* species and chlamydiae can be demonstrated in smears using the modified Ziehl-Neelsen stain. The fluorescent antibody staining method gives rapid specific identification of bacterial pathogens in smears and cryostat tissue sections.

The culture medium, atmospheric conditions and other requirements essential for bacterial isolation are determined by the suspect bacterium. Routine isolation of many pathogens involves inoculation of blood agar and MacConkey agar plates followed by incubation for 24 to 48 hours. Media used in diagnostic bacteriology are indicated in Table 3.2.

Plates should be inoculated using a streaking technique which facilitates growth of isolated colonies. This is an essential step for the identification of pathogens in clinical specimens which may contain microbial contaminants. Definitive identification of a potential pathogen involves subculture of an isolated colony to obtain a pure growth which can then be subjected to biochemical or other tests. Morphological characteristics and biochemical tests allow presumptive identification of a bacterial pathogen. Biochemical tests relate to the catabolic activities of bacteria and an indicator system is usually employed to demonstrate utilization of a particular substrate.

Immunological techniques such as fluorescent antibody staining can be used for identifying bacterial pathogens.



Serotyping is based on the immunological identification of surface antigens on pathogens such as *Escherichia coli* and *Pasteurella multocida*.

The fact that a particular bacteriophage (phage) is specific for a limited number of susceptible strains of bacteria allows differentiation by phage typing. This method is commonly used to differentiate isolates of *Staphylococcus aureus* and also serotypes of *Salmonella* Typhimurium.

Selected molecular techniques can be used for the detection of pathogenic bacteria. The main molecular biological techniques for pathogen detection are nucleic acid hybridization and the polymerase chain reaction. Restriction endonuclease analysis and gene probes are two methods employed for epidemiological investigations. Restriction endonucleases can be used to cleave chromosomal or plasmid DNA to generate fragments which can then be separated by gel electrophoresis. Analysis of the resulting electrophoretic patterns allows comparison of isolates.

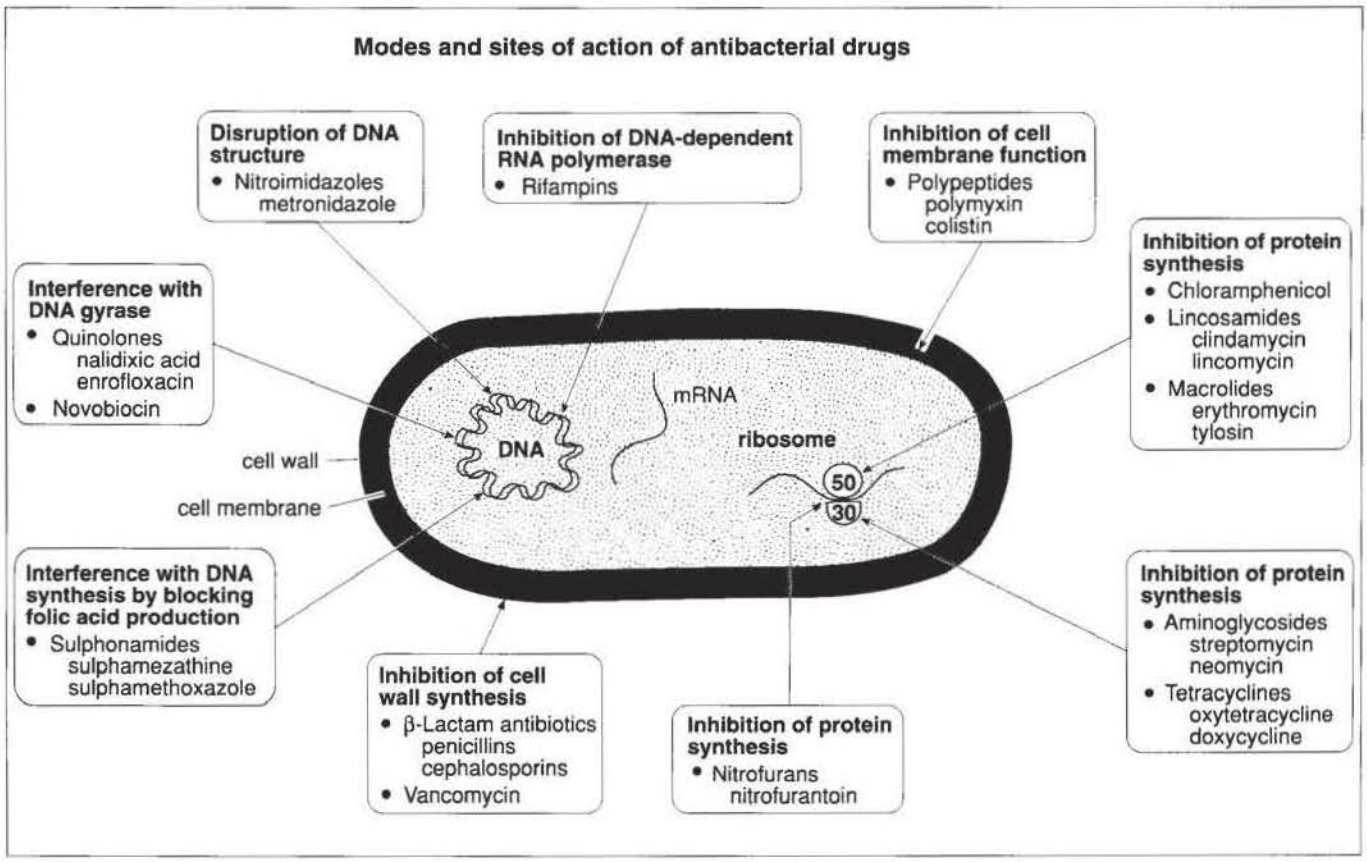
Table 3.1 Routine staining methods for bacteria.

Method	Comments
Gram stain	Widely used for the routine staining of bacteria in smears. Gram-positive bacteria are stained blue by the crystal violet which is retained in their cell walls despite decolourization. In contrast, Gram-negative bacteria, which do not retain the crystal violet, are counterstained red
Giemsa	Useful for demonstrating *Dermatophilus congolensis*, rickettsiae and *Borrelia* species, which stain blue
Dilute carbol fuchsin	Especially useful for recognizing *Campylobacter* species, *Brachyspira* species and *Fusobacterium* species, which stain red
Polychrome methylene blue	Used for the identification of *Bacillus anthracis* in blood smears. The organisms stain blue with distinctive pink capsules
Ziehl-Neelsen stain	Hot concentrated carbol fuchsin which penetrates mycobacterial cell walls is retained after acid-alcohol decolourization. The red-staining bacteria are described as acid-fast or Ziehl-Neelsen-positive
Modified Ziehl-Neelsen stain	Unlike the Ziehl-Neelsen stain, this method employs dilute carbol fuchsin with decolourization by acetic acid

Table 3.2 Laboratory media used for the isolation and presumptive identification of bacterial pathogens.

Medium	Comments
Nutrient agar	A basic medium on which non-fastidious bacteria can grow. Suitable for demonstrating colonial morphology and pigment production; also used for viable counting methods
Blood agar	An enriched medium which supports the growth of most pathogenic bacteria and is used for their primary isolation. Allows the recognition of bacterial haemolysin production
MacConkey agar	A selective medium containing bile which is especially useful for isolation of enterobacteria and some other Gram-negative bacteria. Allows differentiation of lactose fermenters and non-lactose fermenters. Colonies of lactose fermenters and the surrounding medium are pink
Selenite broth, Rappaport-Vassiliadis broth	Selective enrichment media used for the isolation of salmonellae from samples containing other Gram-negative enteric organisms
Edwards medium	A blood agar-based selective medium used for the isolation and recognition of streptococci
Chocolate agar	Heat-treated blood agar which supplies special growth requirements (X and V factors) for the isolation of *Haemophilus* species and for the culture of *Taylorella equigenitalis*
Brilliant green agar	An indicator medium for the presumptive identification of *Salmonella* species. *Salmonella* colonies and surrounding medium have a pink colour

4 Antibacterial agents 1



Antibiotics are low molecular weight microbial metabolites which can kill or inhibit the growth of susceptible bacteria. The therapeutic use of antibiotics depends on their selective toxicity: these drugs kill or inhibit bacterial pathogens without direct toxicity for animals receiving treatment. Individual antibacterial agents are not effective against all pathogenic bacteria. Some are active against a narrow range of bacterial species, while broad spectrum antibiotics such as tetracyclines and chloramphenicol, are active against many species.

The modes and sites of action of antibacterial drugs range from interference with DNA synthesis to inhibition of cell wall synthesis. The major classes of antimicrobial drugs and their modes of action are listed in Table 4.1. Because peptidoglycan is a unique component of bacterial cell walls, antibacterial agents which prevent cross-linking of peptidoglycan chains inhibit cell wall synthesis and are selectively toxic for bacteria. The penicillins and cephalosporins comprise the largest and most important class of antibacterial drugs which inhibit cell wall synthesis. A number of classes of antibacterial agents inhibit protein synthesis. Aminoglycosides bind to 30S ribosomal subunits and affect a number of different steps in protein synthesis. Macrolide antibiotics inhibit protein synthesis by blocking 50S subunit activity. Many antibacterial agents including quinolones, novobiocin, rifampin, nitroimidazoles and sulphonamides inhibit nucleic acid synthesis. The activity of antibacterial drugs is influenced *in vivo* by the site and rate of absorption, the site of excretion and the tissue distribution and metabolism of a particular agent. In addition, antibacterial activity can be affected by interactions between pathogen and drug and between host and pathogen.

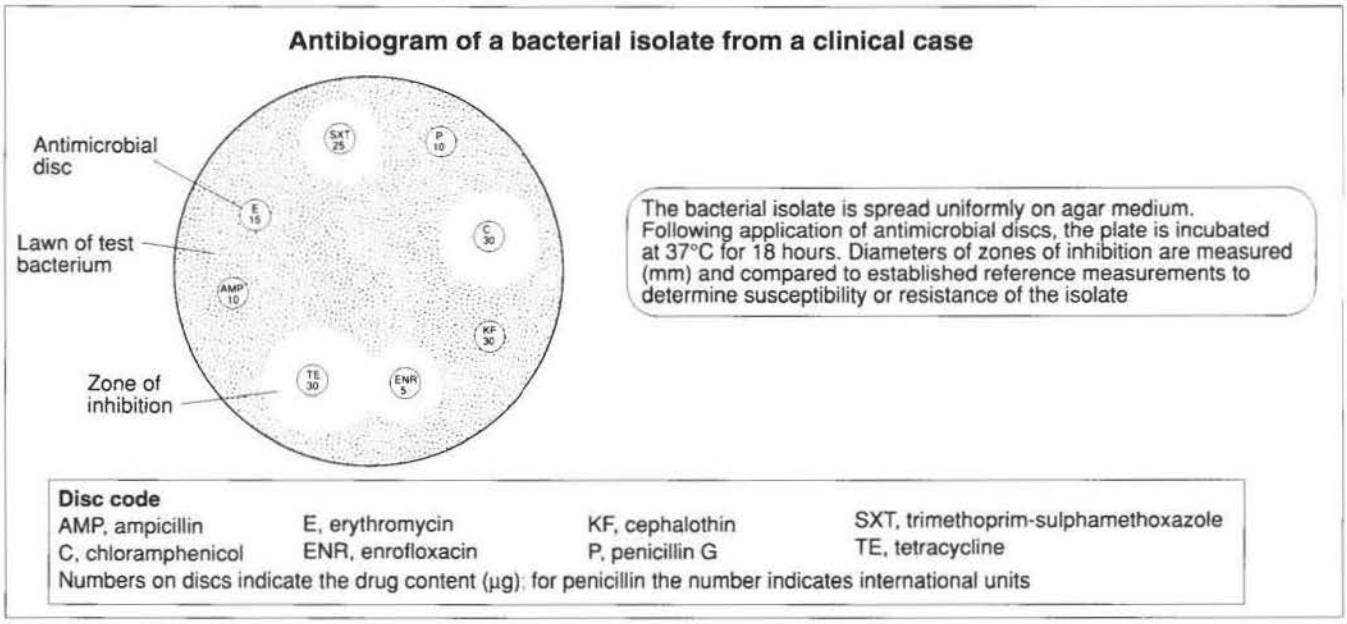
When antibacterial drugs are combined for treatment of disease, the outcome is influenced by the particular combinations employed. If a bacteriostatic drug is combined with a bactericidal drug, antagonism may occur. Bactericidal drugs, particularly the β-lactam antibiotics, are effective against actively dividing cells. If they are combined with a bacteriostatic drug which inhibits bacterial growth, their bactericidal activity may be abolished. Drugs which act synergistically include sulphonamides and trimethoprim, which act at two different sites in the folic acid pathway, and clavulanic acid and penicillin combinations, in which clavulanic acid inhibits β-lactamase activity, preventing inactivation of penicillin.

Antimicrobial drugs can alter the host's immune response and may change the normal flora, particularly on the skin and in the intestinal tract. Disturbance of the normal intestinal flora following therapy for enteric pathogens, such as *Salmonella* species, may allow the development of a prolonged carrier state.

Table 4.1 Major classes of antibacterial drugs and their modes of action.

Antibacterial drug	Mode of action	Effect	Comments
β-Lactam antibiotics Penicillins Cephalosporins	Inhibition of cell wall synthesis	Bactericidal	Low toxicity. Many are inactivated by β-lactamases
Vancomycin	Inhibition of cell wall synthesis	Bactericidal	Used against methicillin-resistant *Staphylococcus aureus*
Polypeptides Polymyxin Colistin	Inhibition of cell membrane function	Bactericidal	Resistance slow to develop. Potentially nephrotoxic and neurotoxic
Nitrofurans Nitrofurantoin	Inhibition of protein synthesis	Bacteriostatic	Synthetic agents with broad-spectrum activity. Relatively toxic
Aminoglycosides Streptomycin Neomycin	Inhibition of protein synthesis. Block 30S ribosomal activity	Bactericidal	Active mainly against Gram-negative bacteria. Ototoxic and nephrotoxic
Tetracyclines Oxytetracycline Doxycycline	Inhibition of protein synthesis. Block 30S ribosomal activity	Bacteriostatic	Formerly used in feed for prophylactic medication. Development of resistance common
Chloramphenicol Florfenicol	Inhibition of protein synthesis. Block 50S ribosomal activity	Bacteriostatic	Use prohibited in food-producing animals in some countries. Potentially toxic
Lincosamides Clindamycin Lincomycin	Inhibition of protein synthesis. Block 50S ribosomal activity	Bactericidal or bacteriostatic	May be toxic in many species. Contraindicated in horses and neonatal animals. Oral administration is hazardous in ruminants
Macrolides Erythromycin Tylosin	Inhibition of protein synthesis. Block 50S ribosomal activity	Bacteriostatic	Active against Gram-positive bacteria. Some macrolides active against mycoplasmal pathogens
Quinolones Nalidixic acid Enrofloxacin	Inhibition of nucleic acid synthesis by blocking DNA gyrase	Bactericidal	Synthetic agents used for treating enteric infections and for intracellular pathogens
Novobiocin	Inhibition of nucleic acid synthesis by blocking DNA gyrase	Bactericidal or bacteriostatic	Often used along with other compatible drugs for treatment of mastitis
Rifampins	Inhibition of nucleic acid synthesis by blocking DNA-dependent RNA polymerase	Bacteriostatic	Antimycobacterial activity; used with erythromycin for treating *Rhodococcus equi* infections
Sulphonamides Sulphamezathine Sulphamethoxazole	Inhibition of nucleic acid synthesis by competitive blocking of para-aminobenzoic acid (PABA) incorporation into folic acid	Bacteriostatic	Synthetic structural analogues of PABA active against rapidly growing bacteria
Trimethoprim	Inhibition of nucleic acid synthesis by combining with the enzyme dihydrofolate reductase	Bacteriostatic	Usually administered with sulphamethoxazole. This combination, referred to as a potentiated sulphonamide, is bactericidal
Nitroimidazoles Metronidazole	Disruption of DNA structure and inhibition of DNA repair	Bactericidal	Particularly active against anaerobic bacteria; also active against some protozoa

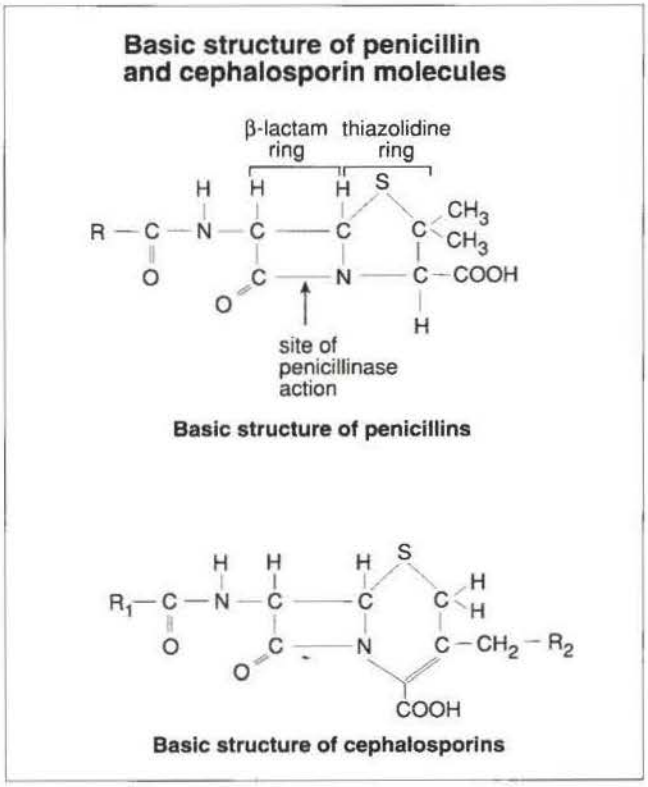
5 Antibacterial agents 2



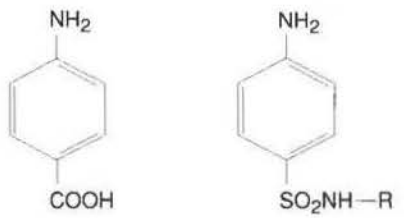
Tests to determine the most suitable antibiotic for effective treatment of a given disease can be conducted on isolates from clinical cases. These tests which are carried out *in vitro* cannot allow for the various factors which may affect antibacterial activity *in vivo*. The results obtained following treatment may not reflect the susceptibility pattern of an isolate as determined in the laboratory. The antibacterial susceptibility tests available include broth dilution, disc diffusion, agar gradient and some automated methods. The Kirby-Bauer disc diffusion method is a flexible and relatively inexpensive technique which is commonly used in diagnostic laboratories. Using this disc diffusion method, susceptibility to an antibacterial drug indicates that the infection caused by the bacterium may respond to treatment if the drug reaches therapeutic levels in the affected tissues.

The mode of action of β-lactam antibiotics involves binding to cell receptors known as penicillin-binding proteins. In addition to interfering with transpeptidation, many of these drugs promote autolysin activity causing cell lysis. Bacteria which produce β-lactamases are resistant to β-lactam antibiotics. β-Lactamases cleave the β-lactam ring, rendering the antibiotic ineffective. These enzymes may be plasmid-mediated, as in staphylococci, or they may be chromosomally encoded, as in Gram-negative bacteria. Sulphonamides interfere with the formation of folic acid, an essential precursor for nucleic acid synthesis. Their action relates to their structural similarity to para-aminobenzoic acid. When present at sufficient concentrations, sulphonamides are utilized by the enzyme dihydropteroate synthetase instead of para-aminobenzoic acid, forming non-functional analogues of folic acid.

Resistance to antibacterial drugs is an important problem in both animals and humans. The widespread and sometimes indiscriminate use of these drugs results in the selection of bacteria which are inherently resistant. Not only may these resistant bacteria become the predominant species in a population but they may also transfer genetic material to other



Basic structure of sulphonamides, analogues of para-aminobenzoic acid



Para-aminobenzoic acid **Basic structure of sulphonamides**

bacterial species which then acquire resistance. Antibacterial drug resistance can be encoded either in the bacterial chromosome or on plasmids (Table 5.1). Resistance genes can be transferred between bacteria through transduction, conjugation, transposable elements or transformation. Resistance to an antibacterial agent often results in cross-resistance to other agents in the same class. Plasmids and transposable elements often mediate multiple resistance in which organisms become resistant to a number of drugs from different classes. This type of resistance can be transferred rapidly between different bacterial species and genera creating multi-drug resistant isolates such as *Salmonella* Typhimurium DT104.

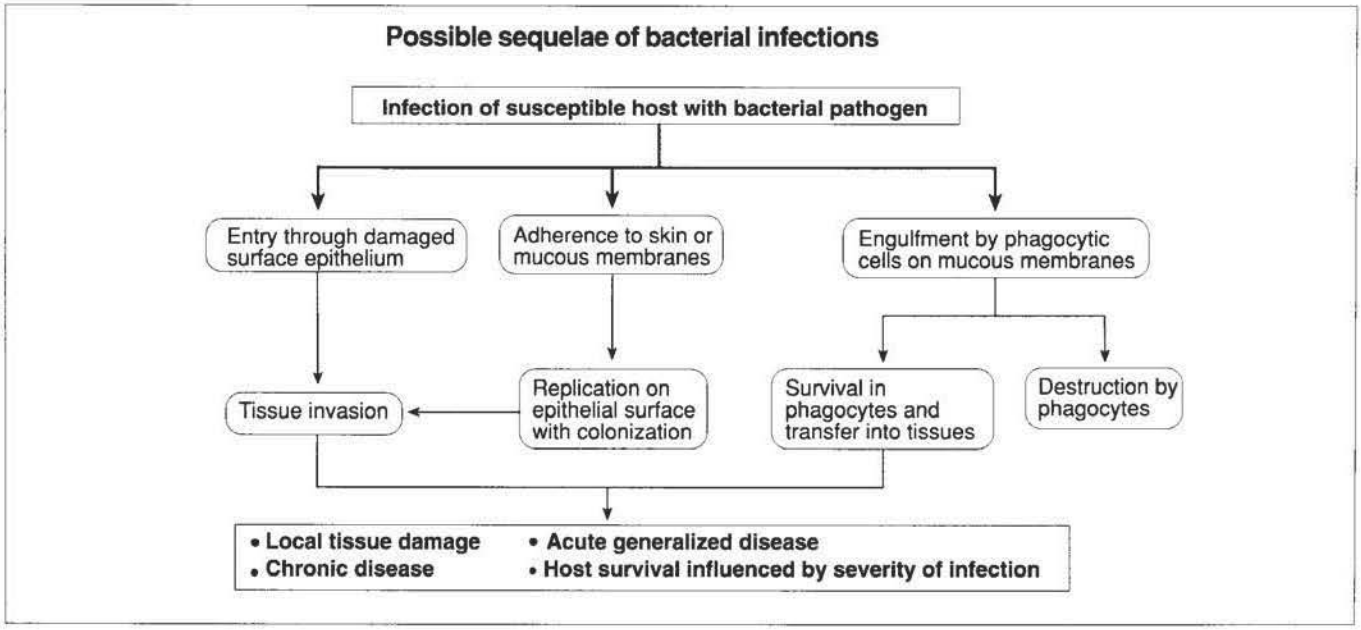
Mechanisms producing resistance to antibacterial drugs include production of enzymes by bacteria which destroy or inactivate the drug and reduction of bacterial cell permeability. Bacteria may also develop alternative metabolic pathways to those inhibited by the drug. The antibiotic may be eliminated from the cell, or the target site of the drug may be structurally altered.

Antibacterial resistance is widespread and control measures in a country may not be effective if resistant bacteria in food or in the normal flora of animals or humans are imported from countries with less stringent controls. Effective surveillance systems for collecting data on resistant organisms should be established at local, national and international levels. The supply and use of antibacterial drugs should be closely monitored to allow evaluation of the risks and benefits of therapy. There should be strict adherence to the recommended therapeutic dose for the prescribed period of time. Adherence to drug withdrawal periods following treatment of food-producing animals should be strictly enforced. Antimicrobial agents should not be used for growth promotion and greater reliance should be placed on improved hygiene measures, disinfection and vaccination for the prevention and control of infectious diseases in animals.

Table 5.1 Antibacterial drug resistance.

Drug	Target	Examples of resistant bacteria / Genetic basis	Comments
Erythromycin	Ribosomal protein	*Staphylococcus aureus* / Chromosomal-based	Ribosomes unaffected by drug action due to structural change
Streptomycin	Ribosomal protein	*Enterobacteriaceae* / Chromosomal-based	Mutation results in altered ribosome
Tetracycline	Ribosomal protein	*Enterobacteriaceae* / Plasmid-mediated	Ribosome protection proteins produced
	Transport mechanisms	*Enterobacteriaceae* / Plasmid-mediated	Decreased absorption or development of energy-dependent efflux mechanism
Rifampin	DNA-dependent RNA polymerase	*Enterobacteriaceae* / Chromosomal-based	Mutation results in structurally altered enzyme
Fluoroquinolones	DNA gyrase Topoisomerase	Gram-positive, Gram-negative / Chromosomal-based	Mutation results in structurally altered enzyme
	Cell membrane	*Enterobacteriaceae* / Chromosomal-based	Decreased permeability
β-Lactam antibiotics	Penicillin-binding proteins (PBP)	*Staphylococcus aureus* / Chromosomal-based	Decreased affinity of PBP for drug
	Penicillin-binding proteins	*Enterobacteriaceae* / Chromosomal-based	Outer membrane of most Gram-negative bacteria inherently impermeable to drug
	Penicillin-binding proteins	*Staphylococcus aureus*, *Enterobacteriaceae* / Plasmid- or chromosomal-based	Enzymatic degradation of drug by β-lactamases
Chloramphenicol	Peptidyltransferase	*Staphylococcus* species, *Streptococcus* species / Plasmid- or chromosomal-based	Inactivation of drug by a specific acetyltransferase
Sulphonamides	Dihydropteroate synthetase	*Enterobacteriaceae* / Plasmid- or chromosomal-based	New folic acid synthetic pathway employing sulphonamide-resistant enzyme

6 Bacterial infections



Although most bacteria are saprophytes which grow on organic matter in the environment, a small number, referred to as bacterial pathogens, produce infection and disease in animals and humans. The development and severity of infections with many pathogenic bacteria are influenced by host-related determinants such as physiological status and immune competence.

Animals may be exposed to infection from exogenous or endogenous sources. Exogenous infections occur after direct or indirect transmission from an infected animal or from the environment. Endogenous infections can be caused by commensal bacteria when an animal is subjected to stressful environmental factors. Infections can be acquired by a number of routes. In exogenous infections, pathogens may enter the host through the skin, the conjunctiva or the mucous membranes of the respiratory, gastrointestinal or urogenital tracts. Other possible routes of entry include the teat canal and the umbilicus.

The virulence of a bacterium relates to its ability to invade and produce disease in a normal animal. Highly virulent organisms produce serious disease or death in many affected animals whereas bacteria of low virulence rarely produce serious illness. Factors which influence the outcome of interactions between host and pathogen are illustrated.

Avoidance of defence mechanisms is essential for successful invasion of the host by pathogens. Some of the mechanisms which assist bacterial survival in animals are presented in Table 6.1. Certain bacteria remain at the site of primary infection with local extension only. This localized invasion may be facilitated through breakdown of host tissues by collagenases, lipases, hyaluronidases and fibrinolysin produced by bacteria. In the bloodstream, bacteria can be carried throughout the body. In bacteraemia, bacteria are present transiently in the blood stream without replication. In septicaemia, pathogenic organisms multiply and persist in the bloodstream, producing systemic disease.

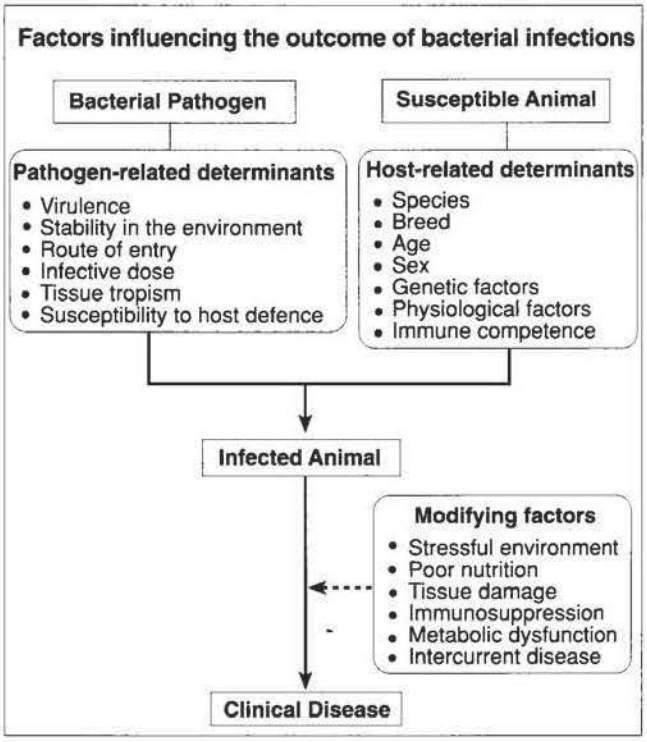


Table 6.1 Mechanisms which assist bacterial survival in the host.

Mechanism	Comments
O antigen polysaccharide chain	Length of polysaccharide chain hinders binding of the membrane attack complex of complement to the outer membrane of many Gram-negative bacteria
Capsular antigen	Incorporation of sialic acid by some Gram-negative bacteria has an inhibitory effect on complement activity
Capsule production	Antiphagocytic role in many bacteria
M protein production	Antiphagocytic activity in *Streptococcus equi*
Production of Fc-binding proteins	Staphylococci and streptococci produce proteins which bind to the Fc region of IgG and prevent interaction with the Fc receptor on membranes of phagocytes
Production of leukotoxins	Cytolysis of phagocytes by toxins produced by *Mannheimia haemolytica*, *Actinobacillus* species and other pathogenic bacteria
Interference with phagosome-lysosome fusion	Allows the survival of pathogenic mycobacteria within phagocytes
Escape from phagosomes	Survival mechanism used by *Listeria monocytogenes* and rickettsiae
Resistance to oxidative damage	Allows survival of salmonellae and brucellae within phagocytes
Antigenic mimicry of host antigens	Adaptation of surface antigens by *Mycoplasma* species to avoid recognition by the immune system
Antigenic variation of surface antigens	Permit survival of *Mycoplasma* species and borrelliae despite the host's immune response to these pathogens
Coagulase production	Conversion of fibrinogen to fibrin by *Staphylococcus aureus* can isolate site of infection from effective immune responses

Bacteria can damage host tissues directly through the effect of exotoxins and endotoxins. Bacterial exotoxins and endotoxins differ in their structures and modes of action (Table 6.2). Exotoxins are produced by Gram-positive and Gram-negative bacteria. The effects of exotoxins, which include cell membrane damage or interference with protein synthesis, are summarized in Box 6.1. Endotoxins of Gram-negative bacteria contain a hydrophobic glycolipid (lipid A) and a hydrophilic polysaccharide composed of a core oligosaccharide and an O-polysaccharide (O antigen). The toxicity of this complex lipopolysaccharide molecule resides in the lipid A portion. The effects of endotoxins are summarized in Box 6.2.

Some individual pathogens tend to produce a predictable clinical picture following infection of a susceptible animal. Anthrax in ruminants is invariably peracute and fatal. In contrast, infections with bacteria such as *Salmonella* Dublin in cattle may produce many different forms of disease. Bacterial infections can be conveniently categorized as acute, subacute, chronic or persistent. Acute infections usually have a short clinical course and the invading bacteria are often cleared from the body by the host's immune response. Chronic infections tend to occur when the host fails to eliminate the pathogen. Persistence occurs in certain sites such as the urinierous tubules and the CNS in which the effects of cell-mediated and humoral immunity are minimal.

Box 6.1 Effects of exotoxins

- Cell membrane damage
  - — Enzymatic digestion
  - — Formation of pores
- Interference with protein synthesis
- Elevation of cAMP levels
- Disruption of functions relating to nervous tissue
- Digestion of components of interstitial tissue: collagen, elastin, hyaluronic acid

Box 6.2 Effects of endotoxins

- Interaction with polymorphonuclear and mononuclear phagocytes, platelets and B lymphocytes
- Release of interleukin-1, leading to fever
- Activation of complement, promoting inflammatory changes

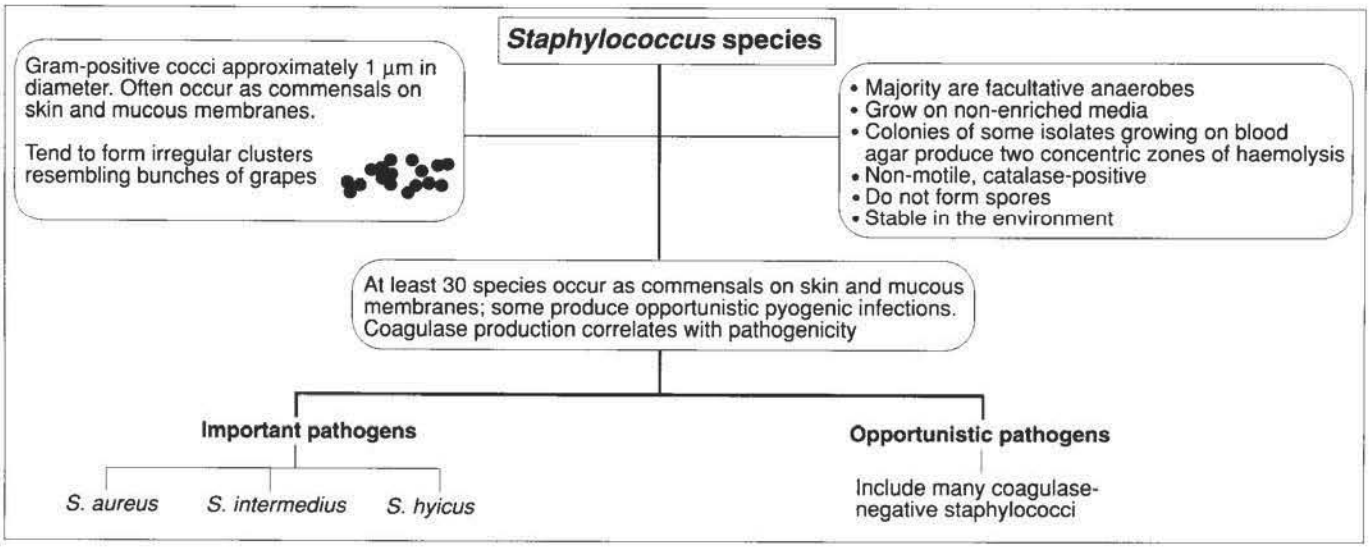
Table 6.2 Comparison of exotoxins and endotoxins.

Exotoxins	Endotoxins
Produced by live bacteria, both Gram-positive and Gram-negative	Component of the cell wall of Gram-negative bacteria released following cell death
Proteins, usually of high molecular weight	Lipopolysaccharide complex containing lipid A, the toxic component
Heat labile	Heat stable
Potent toxins, usually with specific activity; not pyrogenic. Highly antigenic; readily converted into toxoids which induce neutralizing antibodies	Toxins with moderate, non-specific generalized activity; potent pyrogens, weakly antigenic; not amenable to toxoid production. Neutralizing antibodies not associated with natural exposure
Synthesis determined extrachromosomally	Encoded in chromosome

Section II

Pathogenic Bacteria

7 *Staphylococcus* species



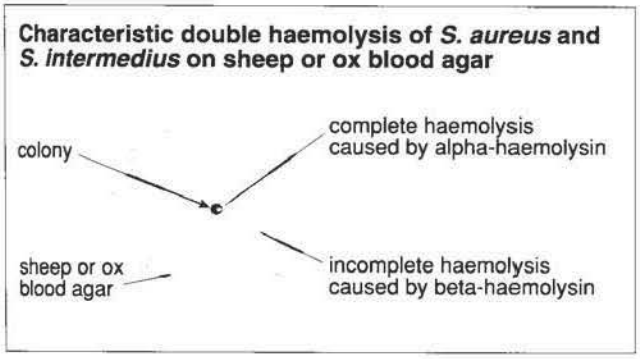
Staphylococci are Gram-positive cocci, approximately 1 μm in diameter, which form irregular clusters resembling bunches of grapes. They occur as commensals on skin and mucous membranes; some act as opportunistic pathogens causing pyogenic infections. They are comparatively stable in the environment. The coagulase-positive *S. aureus* subsp. *aureus* (referred to as *S. aureus*) and *S. intermedius* and the coagulase-variable *S. hyicus* are important pathogens of domestic animals. Coagulase, which converts fibrinogen in plasma to fibrin, is a virulence factor of these organisms which correlates with pathogenicity. Coagulase-negative staphylococci are usually of low virulence. In clinical specimens, *Staphylococcus* species must be differentiated from *Streptococcus* species and from *Micrococcus* species. Staphylococci are generally catalase-positive and streptococci catalase-negative. *Staphylococcus* species are usually categorized by their colonial appearance, haemolytic pattern and biochemical profiles.

Because staphylococci are pyogenic bacteria, they often cause suppurative lesions. Minor trauma or immunosuppression may predispose to the development of infection. Virulence factors of *S. aureus* and their pathogenic effects are indicated in Table 7.1. Although some virulence factors are plasmid-mediated, most are encoded in the staphylococcal genome. Staphylococcal diseases of importance in domestic animals include mastitis, tick pyaemia, exudative epidermitis, botryomycosis and pyoderma (Table 7.2).

Staphylococcal mastitis, usually caused by *S. aureus*, is a common form of bovine mastitis worldwide. Infection occurs mainly at milking through contaminated milker's hands, teat cup liners and udder cloths. The disease may be subclinical, acute or chronic. Most infections are subclinical. Peracute and gangrenous forms are associated with severe systemic reactions

Table 7.1 Virulence factors, including toxins, of *Staphylococcus aureus* and their pathogenic effects.

Virulence factor	Pathogenic effects
Coagulase	Conversion of fibrinogen to fibrin. Fibrin deposition may shield staphylococci from phagocytic cells
Lipase, esterases, elastase, staphylokinase, deoxyribonuclease, hyaluronidase, phospholipase	Enzymes which contribute to virulence
Protein A	Surface component which binds Fc portion of IgG and inhibits opsonization
Leukocidin	Cytolytic destruction of phagocytes of some animal species
Alpha-toxin (alpha-haemolysin)	The major toxin in gangrenous mastitis. It causes spasm of smooth muscle and is necrotizing and potentially lethal
Beta-toxin (beta-haemolysin)	A sphingomyelinase which damages cell membranes
Exfoliative toxins	Responsible for desquamation in staphylococcal 'scalded skin syndrome' in humans
Enterotoxins	Heat-stable toxins associated with staphylococcal food poisoning in humans



and can be life-threatening. In chronic or subclinical staphylococcal mastitis episodes of bacterial shedding from affected quarters occur along with elevated somatic cell counts. Bacterial multiplication occurs principally in the collecting ducts and to a limited extent, in the alveoli. Influx of phagocytic cells may lead to abscess formation and fibrosis, which further limits effective clearance of the organisms and also interferes with antibiotic penetration during treatment. Although some *S. aureus* intramammary infections are cleared by immune mechanisms, the majority become chronic, low-grade or subclinical, resulting in substantial production losses.

Tick pyaemia, an infection of lambs with *S. aureus*, is confined to hill-grazing regions of Britain and Ireland where there are suitable habitats for the tick *Ixodes ricinus*. Lambs can carry *S. aureus* on their skin and nasal mucosa and infection occurs through minor skin trauma, including tick bites. Tick pyaemia is characterized either by septicaemia and rapid death or by localized abscess formation in many organs. Clinical manifestations include arthritis, posterior paresis and ill-thrift. Microscopic demonstration of the bacteria in pus, followed by isolation and identification of *S. aureus* from lesions, is confirmatory. Treatment is of limited value in severely affected lambs. Efforts should be directed at control within the flock, and tick-control measures such as dipping should be introduced.

Exudative epidermitis, caused by *S. hyicus*, occurs worldwide in sucklers and weaned pigs up to three months of age. This highly contagious disease is characterized by widespread excessive sebaceous secretion, exfoliation and exudation on the skin surface. Affected pigs, which are anorexic, depressed and febrile, have an extensive, non-pruritic dermatitis with a greasy exudate. Morbidity rates range from 20% to 100% and mortality rates can reach 90% in severely affected litters. Predisposing stress factors include agalactia in the sow, intercurrent infections and weaning. The organism probably enters the skin of young pigs through minor abrasions such as bite wounds. *Staphylococcus hyicus* can be isolated from the vaginal mucosa and skin of healthy sows. Isolation and identification of *S. hyicus* from dermal lesions is confirmatory.

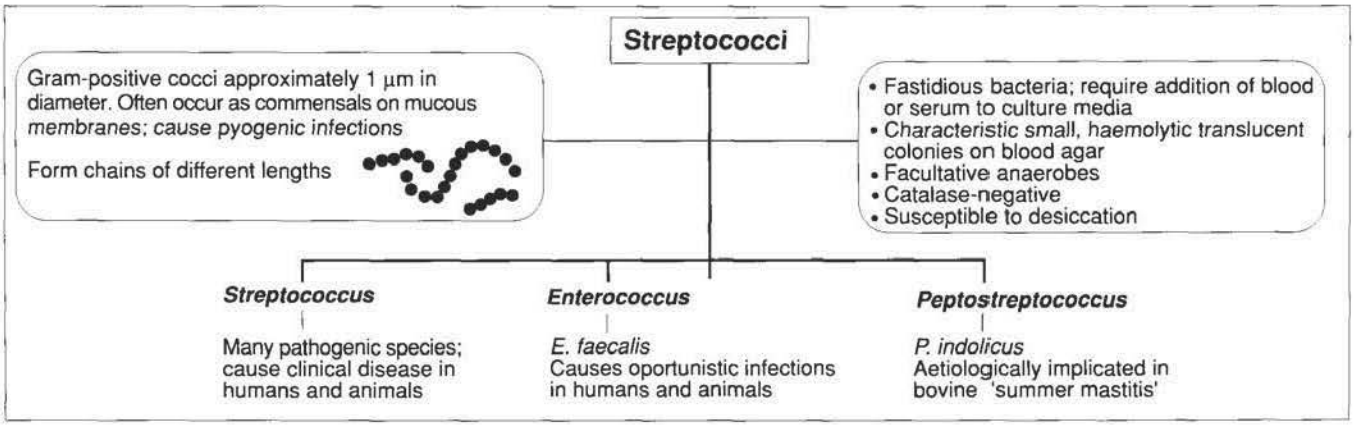
Table 7.2 Coagulase-positive staphylococci and their clinical importance.

Species	Hosts	Clinical conditions
Staphylococcus aureus	Cattle	Mastitis, udder impetigo
	Sheep	Mastitis Tick pyaemia (lambs) Benign folliculitis (lambs) Dermatitis
	Goats	Mastitis Dermatitis
	Pigs	Botryomycosis of mammary glands Impetigo on mammary glands
	Horses	Scirrhous cord (botryomycosis of the spermatic cord), mastitis
	Dogs, cats	Suppurative conditions similar to those caused by *S. intermedius*
	Poultry	Arthritis and septicaemia in turkeys Bumblefoot Omphalitis in chicks
S. intermedius	Dogs	Pyoderma, endometritis, cystitis, otitis externa, and other suppurative conditions
	Cats	Various pyogenic conditions
S. hyicus	Pigs	Exudative epidermitis (greasy pig disease) Arthritis

Botryomycosis, a chronic suppurative granulomatous condition often caused by *S. aureus*, can occur in horses after castration, due to infection of the stump of the spermatic cord. The lesion is composed of a mass of fibrous tissue containing foci of pus and sinus tracts.

Staphylococcus intermedius is commonly isolated from pyoderma, otitis externa and other suppurative conditions including mastitis, endometritis, cystitis, osteomyelitis and wound infections in dogs and cats. Occasionally, similar suppurative conditions are caused by *S. aureus*.

8 Streptococci



Streptococci are a group of bacteria which can cause pyogenic infections in many animal species. These Gram-positive cocci form chains of different lengths. They are fastidious bacteria and require the addition of blood or serum to culture media. *Streptococcus* species are non-motile, facultative anaerobes which are catalase-negative. *Enterococcus* species are enteric streptococci found in the intestine of humans and animals.

Three laboratory procedures are used for differentiating streptococci: type of haemolysis, Lancefield grouping and biochemical testing. On sheep or ox blood agar, beta-haemolysis refers to complete haemolysis indicated by clear zones around colonies. Alpha-haemolysis is partial haemolysis indicated by greenish or hazy zones around colonies. Lancefield grouping is a serological method of classification based on group-specific C-substance (polysaccharide) in the cell wall. Test methods used include ring precipitation and latex agglutination. Pyogenic streptococci are associated with abscess formation, other suppurative conditions and septicaemias. Beta-haemolytic streptococci are usually more pathogenic than those producing alpha-haemolysis. Virulence factors include enzymes and exotoxins such as streptolysins (haemolysins), hyaluronidase, DNase, streptokinase and proteases. Polysaccharide capsules, which are major virulence factors of *S. pyogenes* and some strains of *S. equi*, are antiphagocytic. Identification criteria for streptococcal isolates include the presence of small, translucent colonies, some of which may be mucoid. Chains of Gram-positive cocci which are catalase-negative and the type of haemolysis produced on blood agar are indicative of streptococcal organisms. Definitive identification requires a biochemical test profile of the isolate and Lancefield grouping.

Streptococci are often commensals on mucous membranes and, consequently, many streptococcal infections are opportunisitic. Infections may be primary, as in strangles, or secondary as in streptococcal pneumonia following viral infection. Lymph nodes, genital tract or mammary glands may become infected. Strangles, porcine streptococcal meningitis and bovine streptococcal mastitis are important specific infections. Vaccines for the control of streptococcal infections are usually ineffective. The clinical consequences of streptococcal infections are listed in Table 8.1.

Strangles is a highly contagious disease of horses caused by *Streptococcus equi*. It is a febrile disease involving the upper respiratory tract with abscessation of regional lymph nodes. Outbreaks of disease most commonly occur in young horses. Assembling horses at sales, shows and race courses increases the risk of acquiring infection. Transmission is via purulent exudates from the upper respiratory tract or from discharging abscesses. Infected animals may shed *S. equi* for at least four weeks after development of clinical signs. The incubation period is up to 6 days and the course of uncomplicated disease is about 10 days. There is a high fever, depression and anorexia followed by oculonasal discharge that becomes purulent. Characteristically, the submandibular nodes are affected and they may eventually rupture, discharging purulent, highly infectious material. Guttural pouch empyema is a common finding. The

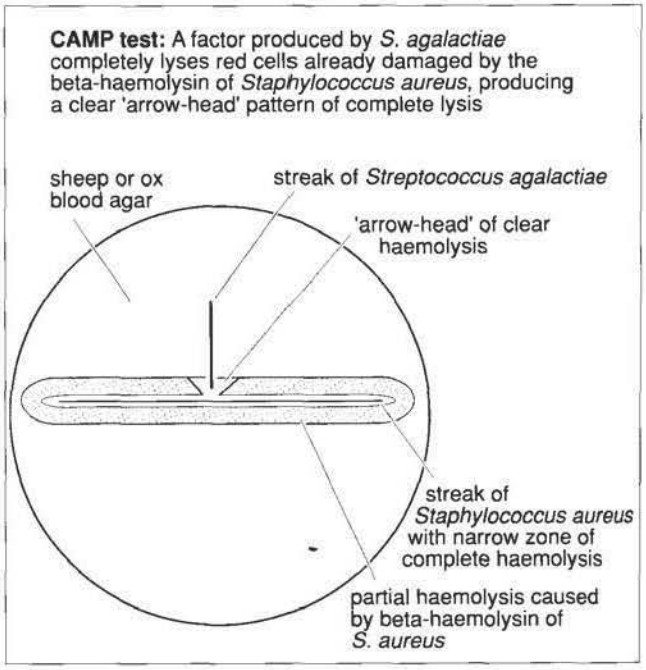


Table 8.1 Pathogenic streptococci, their habitats, hosts and consequences of infection.

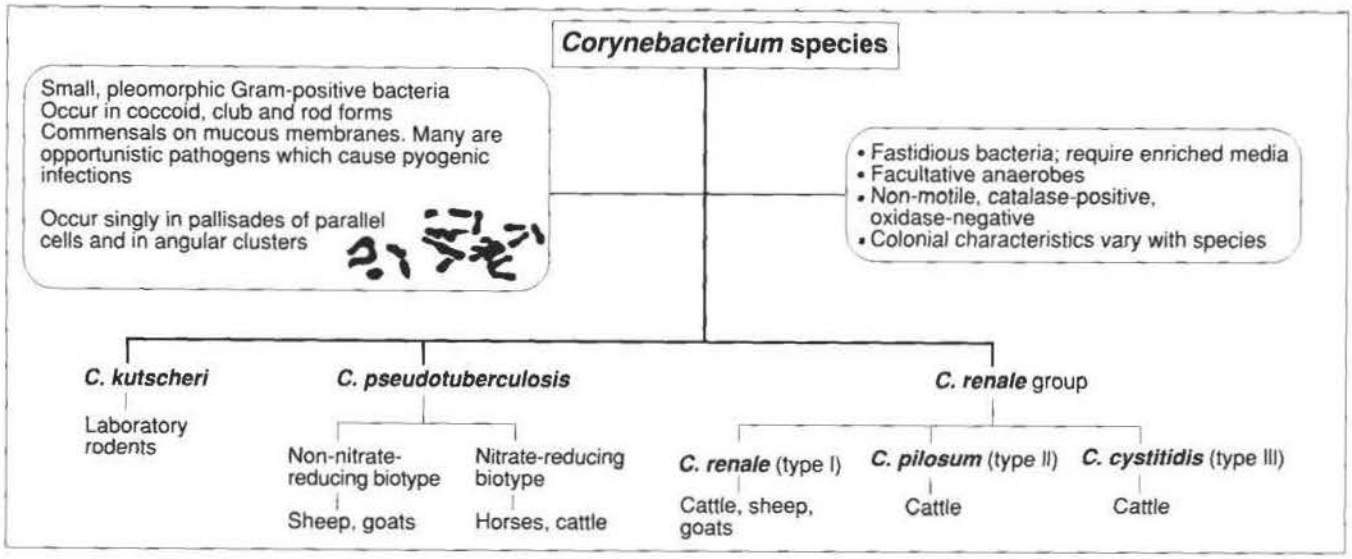
Species	Lancefield group	Haemolysis on blood agar	Hosts	Consequences of infection	Usual habitat
S. pyogenes	A	β	Humans	Scarlet fever, septic sore throat, rheumatic fever	Mainly upper respiratory tract
S. agalactiae	B	β (α, γ)	Cattle, sheep, goats	Chronic mastitis	Milk ducts
			Humans, dogs	Neonatal septicaemia	Vagina
S. dysgalactiae	C	α (β, γ)	Cattle	Acute mastitis	Buccal cavity, vagina, environment
			Lambs	Polyarthritis	
S. equi (*S. equi* subsp. *equi*)	C	β	Horses	Strangles, suppurative conditions, purpura haemorrhagica	Upper respiratory tract, guttural pouch
S. zooepidemicus (*S. equi* subsp. *zooepidemicus*)	C	β	Horses	Mastitis, pneumonia, navel infections	Mucous membranes
			Cattle, lambs, pigs, poultry	Suppurative conditions, septicaemia	Skin, mucous membranes
S. suis	D	α (β)	Pigs	Septicaemia, meningitis, arthritis, bronchopneumonia	Tonsils, nasal cavity
			Humans	Septicaemia, meningitis	
S. canis	G	β	Carnivores	Neonatal septicaemia, suppurative conditions, toxic shock syndrome	Vagina, anal mucosa
S. uberis	Not assigned	α (γ)	Cattle	Mastitis	Skin, vagina, tonsils

morbidity may be up to 100% and mortality is usually less than 5%. Following outbreaks of the disease, buildings and equipment should be cleaned and disinfected.

Streptococcus suis is recognized worldwide as a cause of significant losses in the pig industry. It is associated with meningitis, arthritis, septicaemia and bronchopneumonia in pigs of all ages and with sporadic cases of endocarditis, neonatal deaths and abortion. At least 34 serotypes of varying virulence have been recognized. About 70% of *S. suis* isolates belong to serotypes 1 to 9. Of these, serotype 2 is the most prevalent serotype, with carrier rates up to 90%. This serotype is associated with meningitis in both pigs and humans. Asymptomatic pigs harbour *S. suis* in tonsillar tissue. Disease outbreaks are most common in intensively-reared pigs when they are subjected to overcrowding, poor ventilation and other stress factors. Sows carrying the organisms can infect their litters. Meningitis, which is often fatal, is characterized by fever, tremors, incoordination, opisthotonos and convulsions. As these bacteria tend to become endemic in a herd, eradication is not feasible. Improved husbandry methods may decrease the prevalence of clinical disease.

Streptococcus agalactiae, *S. dysgalactiae* and *S. uberis* are the principal pathogens involved in streptococcal mastitis. Following introduction into the mammary gland, *S. agalactiae* multiplies and invades the lactiferous ducts. An influx of neutrophils into the mammary gland follows and the inflammatory reaction results in blockage of teat ducts and atrophy of secretory tissue. These inflammatory cycles occur periodically with progressive loss of secretory tissue. *Streptococcus dysgalactiae*, which is found in the buccal cavity and genitalia and on the skin of the mammary gland, causes acute mastitis. A number of bacteria which occur in the environment, including *S. uberis*, can cause mastitis. Contamination of teat ends is a major predisposing factor in the development of this form of mastitis. Differentiation of the mastitis-producing streptococci is based on the type of haemolysis produced on blood agar, aesculin hydrolysis in Edwards medium, identification of the Lancefield group to which they belong and the results of the CAMP test. Both *S. dysgalactiae* and *S. uberis* produce alpha-haemolysis while *S. agalactiae* produces beta-haemolysis. Aesculin is hydrolysed by *S. uberis*, while *S. agalactiae* gives a positive CAMP test result.

9 *Corynebacterium* species and *Rhodococcus equi*



Corynebacterium species

Corynebacterium species are small, pleomorphic Gram-positive bacteria which occur in coccoid, club and rod forms. In stained smears they occur singly, in pallisades of parallel cells and in angular clusters resembling Chinese letters. Most corynebacteria are catalase-positive, oxidase-negative, non-spore-forming facultative anaerobes which require enriched media for growth. Pathogenic corynebacteria are non-motile. Many *Corynebacterium* species are commensals on mucous membranes. Most pathogenic corynebacteria are relatively host-specific and produce identifiable clinical syndromes. The host species and the nature of the disease may suggest the causal agent. Identification critieria include bacterial cell morphology, colonial appearance and biochemical reactions.

Corynebacterium pseudotuberculosis has small, whitish colonies surrounded by a narrow zone of complete haemolysis after incubation for 72 hours. Members of the *C. renale* group produce small non-haemolytic colonies after incubation for 24 hours. Conventional or commercially available biochemical tests can be used to differentiate the corynebacteria. Two biotypes of *C. pseudotuberculosis* are recognized. The ovine/caprine strains lack nitrate-reducing capacity, while the equine/bovine strains usually reduce nitrate. Urease is produced by all pathogenic corynebacteria with the exception of *C. bovis*.

Many corynebacteria are opportunistic pathogens. With the exception of *C. bovis*, these organisms are pyogenic and cause a variety of suppurative conditions in domestic animals. The main diseases caused by infections with *Corynebacterium* species are summarized in Table 9.1.

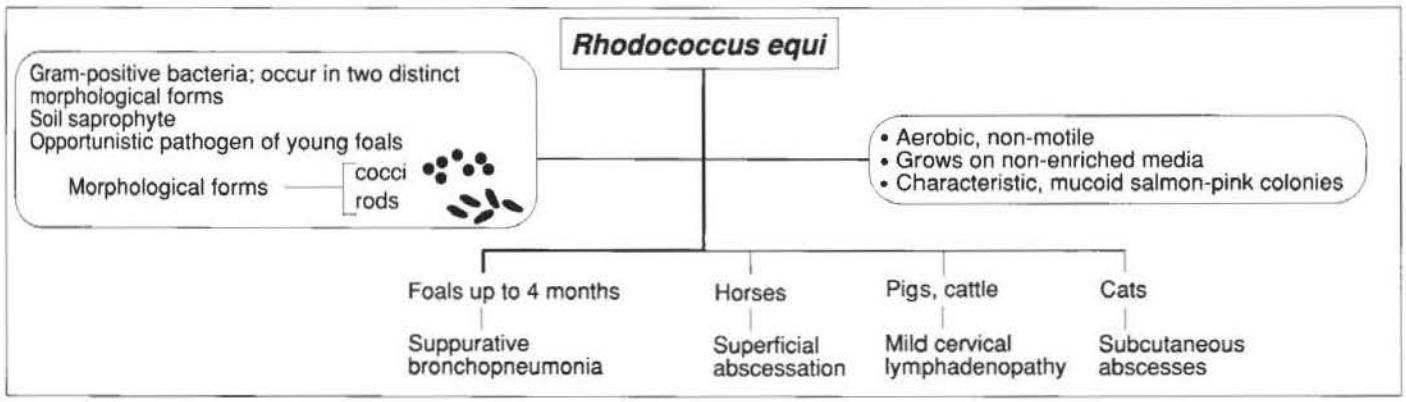
Caseous lymphadenitis, caused by the non-nitrate-reducing biotype of *C. pseudotuberculosis*, is a chronic suppurative condition of sheep, goats and, rarely, cattle. Sheep become infected through contamination of shearing wounds by arthropod bites or from contaminated dips. Infections results in abscessation and enlargement of superficial or internal lymph nodes. The incubation period is about three months. The disease, which is prevalent in Australia, New Zealand, the Middle East, Asia, Africa and parts of North and South America, is being reported more frequently in Britain and other European countries. Ill-thrift may be evident in affected animals and the disease invariably results in condemnation of carcases and devaluation of hides. Infection is spread by pus from ruptured abscesses and from nasal and oral secretions. The organism can survive in the environment for several months. Affected lymph nodes are enlarged and exhibit characteristic encapsulated abscesses which have an 'onion ring' appearance on cut surfaces. Abscess material is caseous, initially greenish and later putty-coloured. The visceral form of the disease may not be detectable antemortem. Goats usually develop the superficial form of the disease. The disease may be suspected on clinical grounds or at postmortem examination. Smears from lesions may reveal Gram-positive coryneform bacteria. Isolation and identification of *C. pseudotuberculosis* from abscess material is confirmatory. Because of the chronic nature of lesions and the ability of the organisms to survive intracellularly, therapy is usually ineffective. Appropriate control measures for individual countries are determined by the prevalence of the disease.

The nitrate-reducing biotype of *C. pseudotuberculosis* causes sporadic cases of ulcerative lymphangitis in horses and cattle. This disease occurs in Africa, the Americas, the Middle East and India. Infection occurs through skin wounds, arthropod bites or by contact with contaminated harness. The condition presents either as lymphangitis of the lower limbs or abscessation in the pectoral region. Diagnosis is based on

Table 9.1 The pathogenic corynebacteria, their hosts, usual habitats and the disease conditions which they produce.

Pathogen	Host	Disease condition	Usual habitat
Corynebacterium bovis	Cattle	Subclinical mastitis	Teat cistern
C. kutscheri	Laboratory rodents	Superficial abscesses, caseopurulent foci in liver, lungs and lymph nodes	Mucous membranes, environment
C. pseudotuberculosis			
Non-nitrate-reducing biotype	Sheep, goats	Caseous lymphadenitis	Skin, mucous membranes, environment
Nitrate-reducing biotype	Horses, cattle	Ulcerative lymphangitis, abscesses	Environment
C. renale group			
C. renale (type I)	Cattle	Cystitis, pyelonephritis	Lower urogenital tracts of cows and bulls
	Sheep and goats	Ulcerative (enzootic) balanoposthitis	Prepuce
C. pilosum (type II)	Cattle	Cystitis, pyelonephritis	Bovine urogenital tract
C. cystitidis (type III)	Cattle	Severe cystitis, rarely pyelonephritis	Bovine urogenital tract
C. ulcerans	Cattle	Mastitis	Human pharyngeal mucosa

isolation and identification of *C. pseudotuberculosis* from lesions. Organisms belonging to the *C. renale* group can be isolated from the vulva, vagina and prepuce of apparently normal cattle. The stress of parturition and the shortness of the urethra in the cow predispose to infection of the urinary tract. Ascending infection from the bladder through the ureters can result in pyelonephritis. Clinical signs of pyelonephritis include fever, anorexia and decreased milk production. Restlessness and kicking at the abdomen may indicate renal pain. Dysuria, an arched back and blood-tinged urine are invariably present. The culture of *C. renale* from urinary deposits, in association with the presence of characteristic clinical signs, is confirmatory.

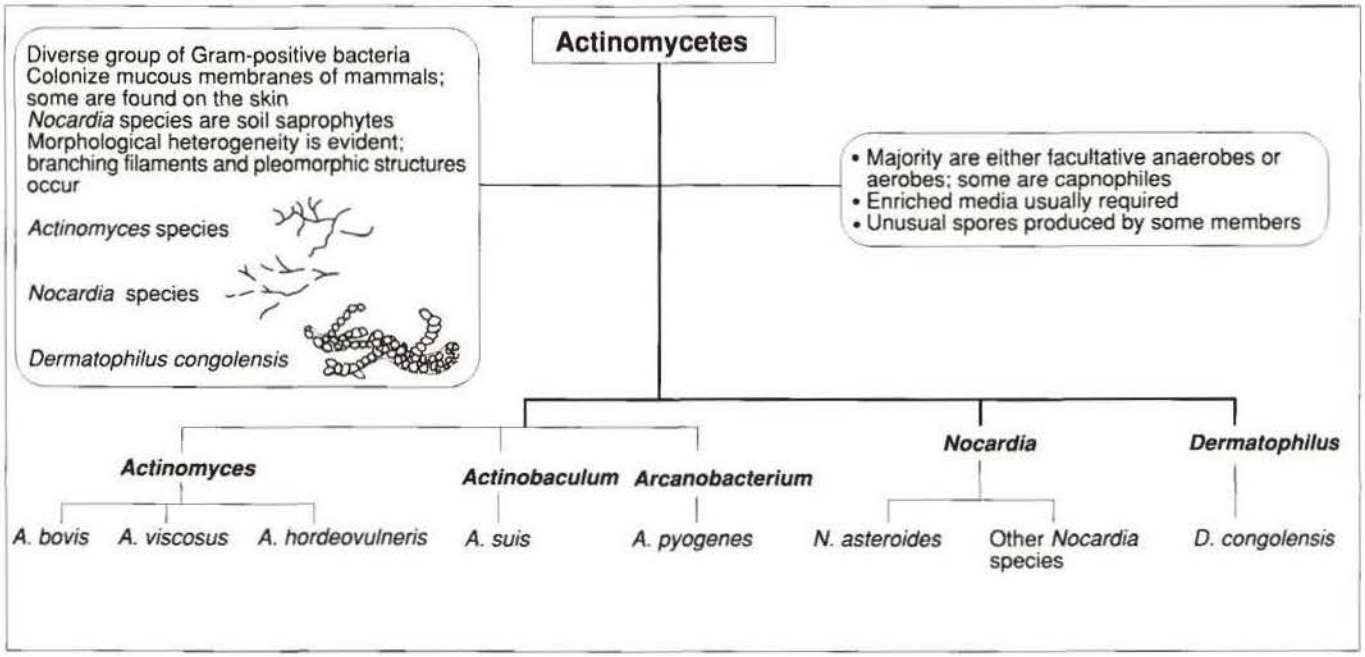


Rhodococcus equi

Rhodococcus equi, formerly called *Corynebacterium equi*, is a Gram-positive, aerobic soil saprophyte which occurs worldwide. It is an opportunisitic pathogen of foals under six months of age. *Rhodococcus equi* grows on non-enriched media and produces characteristic mucoid salmon-pink colonies. Some strains of *R. equi* appear as cocci, and others as rods up to 5 µm in length.

Suppurative bronchopneumonia of foals is the major disease caused by this pyogenic organism. Infection is generally acquired by inhalation of dust contaminated with *R. equi*. The organism is often present in large numbers in the faeces of healthy foals and also in the faeces of older horses. A build-up of *R. equi* can occur on pastures heavily stocked with horses. Acute disease often occurs in one month old foals, with sudden onset of fever, anorexia and signs of bronchopneumonia. In older foals, the disease tends to be insidious and lesions can be well advanced before animals exhibit coughing, dyspnoea, weight loss and characterisitic loud, moist rales on auscultation of the lungs. A history of the disease on the farm, the age of the affected foal and clinical signs may suggest infection with *R. equi*. Culture of *R. equi* from tracheal aspirates and pus from lesions, in association with clinical signs, is confirmatory.

10 Actinomycetes 1



The actinomycetes are a phylogenetically diverse group of Gram-positive bacteria which tend to grow slowly and produce branching filaments. The bacteria in this group which cause disease in domestic animals, belong to the genera *Actinomyces*, *Arcanobacterium*, *Actinobaculum*, *Nocardia* and *Dermatophilus*. Comparative features of actinomycetes of veterinary importance are presented in Table 10.1.

Actinomyces, *Arcanobacterium* and *Actinobaculum* species

Species in these genera are non-motile, non-spore-forming, Gram-positive bacteria which require enriched media for growth. *Arcanobacterium pyogenes* was formerly called *Actinomyces pyogenes* and *Actinobaculum suis* was formerly known by other names. The species of veterinary importance in

Table 10.1 Comparative features of actinomycetes of veterinary importance.

Feature	*Actinomyces* species	*Arcanobacterium pyogenes*	*Actinobaculum suis*	*Nocardia* species	*Dermatophilus congolensis*
Atmospheric growth requirements	Anaerobic or facultatively anaerobic and capnophilic	Facultatively anaerobic and capnophilic	Anaerobic	Aerobic	Aerobic and capnophilic
Aerial filament production	–	–	–	+	–
Modified Ziehl-Neelsen staining	–	–	–	+	–
Growth on Sabouraud dextrose agar	–	–	–	+	–
Usual habitat	Nasopharyngeal and oral mucosae	Nasopharyngeal mucosa of cattle, sheep and pigs	Prepuce and preputial diverticulum of boars	Soil	Skin of carrier animals, scabs from lesions
Site of lesions	Many tissues including bone	Soft tissues	Urinary tract of sows	Thoracic cavity, skin and other tissues	Skin

Table 10.2 Differentiation of *Actinomyces*, *Arcanobacterium* and *Actinobaculum* species of veterinary importance.

Characteristic	*Actinomyces bovis*	*Actinomyces viscosus*	*Actinomyces hordeovulneris*	*Arcanobacterium pyogenes*	*Actinobaculum suis*
Morphology	Filamentous, branching, some short forms	Filamentous, branching, short forms	Filamentous, branching, short forms	Coryneform	Coryneform
Atmospheric requirements	Anaerobic + CO_2	10% CO_2	10% CO_2	Aerobic	Anaerobic
Haemolysis on sheep blood agar	±	–	±	+	±
Catalase production	–	+	+	–	–
Pitting of Loeffler's serum slope	–	–	–	+	–
Granules in pus	'Sulphur granules'	White granules	No granules	No granules	No granules

the group are *Arcanobacterium pyogenes*, *Actinobaculum suis*, *Actinomyces bovis*, *Actinomyces viscosus* and *Actinomyces hordeovulneris*. Apart from *A. hordeovulneris*, pathogenic members of these genera colonize mucous membranes of mammals. Differentiating features of the genera are presented in Table 10.2. Disease conditions produced by *Actinomyces*, *Arcanobacterium* and *Actinobaculum* species in domestic animals are summarized in Table 10.3.

Table 10.3 Disease conditions produced by *Actinomyces*, *Arcanobacterium* and *Actinobaculum* species in domestic animals.

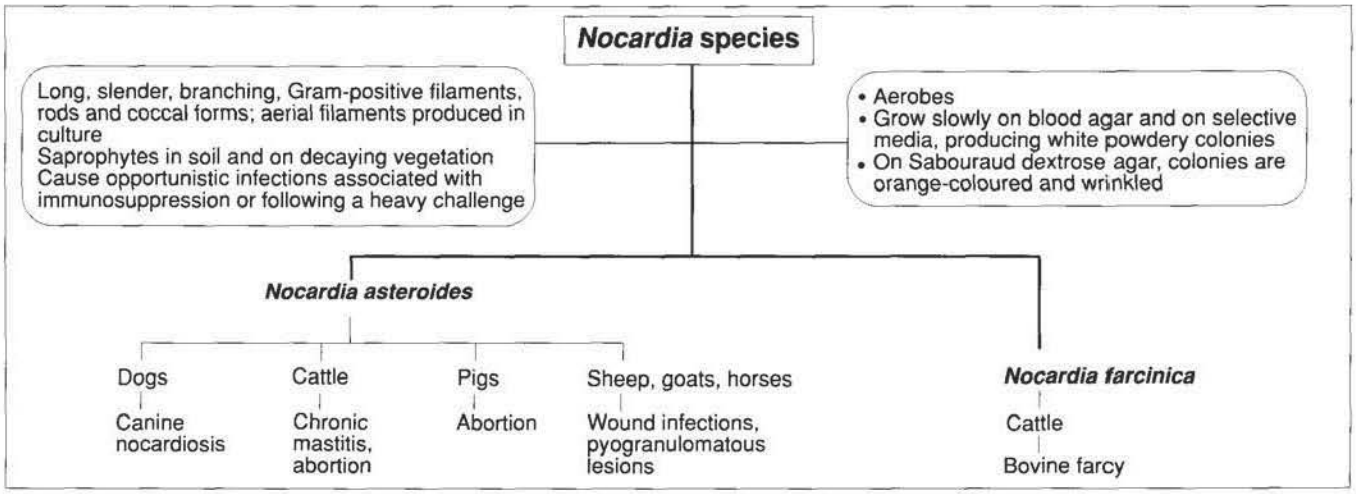
Species	Hosts	Disease conditions
Arcanobacterium pyogenes	Cattle, sheep, pigs	Abscessation, mastitis, suppurative pneumonia, endometritis, pyometra, arthritis, umbilical infections
Actinomyces hordeovulneris	Dogs	Cutaneous and visceral abscessation, pleuritis, peritonitis, arthritis
Actinomyces bovis	Cattle	Bovine actinomycosis ('lumpy jaw')
A. viscosus	Dogs	Canine actinomycosis: — cutaneous pyogranulomas — pyothorax and proliferative pyogranulomatous pleural lesions — disseminated lesions (rare)
	Horses	Cutaneous pustules
	Cattle	Abortion
Actinomyces species (unclassified)	Pigs	Pyogranulomatous mastitis
	Horses	Poll evil and fistulous withers
Actinobaculum suis	Pigs	Cystitis, pyelonephritis

Arcanobacterium pyogenes is a common cause of suppurative lesions in many domestic species worldwide, especially cattle, pigs and sheep. Lymphadenitis, osteomyelitis, peritonitis and neural abscessation are commonly associated with tissue invasion by this pathogen. This organism is also implicated in pyometra, metritis and acute mastitis in dairy cows. In the acute bovine mastitis, referred to as 'summer mastitis' in Britain and Ireland, the anaerobic bacterium *Peptostreptococcus indolicus* is usually associated with *A. pyogenes*. In foot lesions in ruminants, and other mixed infections, *A. pyogenes* also occurs in association with anaerobes. Diagnosis is based on the typical pleomorphic cell morphology in Gram-stained smears from specimens, colonial characteristics and the ability of *A. pyogenes* to pit a Loeffler's serum slope.

Invasion of the mandible and, less commonly, the maxilla of cattle by *A. bovis* causes a chronic rarefying osteomyelitis referred to as 'lumpy jaw'. The organsim is presumed to invade the tissues following trauma to the mucosa from rough feed or through dental alveoli during tooth eruption. A painless swelling of the affected bone enlarges over a period of several weeks. The swelling becomes painful and fistulous tracts, discharging exudate containing 'sulphur granules' with characteristic club colonies, develop. Spread to contiguous soft tissues may occur, but there is minimal involvement of regional lymph nodes. Surgery is the treatment of choice when lesions are small and circumscribed. In advanced cases, surgical treatment may be ineffective.

Porcine cystitis and pyelonephritis, caused by *Actinobaculum suis* affects the urinary tract of pregnant sows. The pathogen, which is transmitted at-coitus, causes a potentially fatal infection. Anorexia, arching of the back, dysuria and haematuria are prominent signs. If both kidneys are extensively damaged, death may result.

11 Actinomycetes 2



Nocardia species and *Dermatophilus congolensis*

Nocardia species

Members of the *Nocardia* species are Gram-positive, aerobic, saprophytic actinomycetes. In smears of exudate from infected tissue, they appear as long, slender, branching filaments with a tendency to fragment into rods and cocci. When cultured, these organisms produce aerial filaments which may form spores. Components of the cell wall render *Nocardia* species partially acid-fast (modified Ziehl-Neelsen-positive). *Nocardia asteroides* is the pathogen of greatest significance in this genus.

Nocardia species are saprophytes found in soil and decaying vegetation. Infection, which is opportunistic, is usually associated with immunosuppression or, alternatively, may follow a heavy challenge. The usual mode of infection is by inhalation but it may also occur through skin wounds or via the teat canal. Virulent strains of *N. asteroides* survive intracellularly. Cell-mediated immunity is essential for protection against infection by this facultative intracellular bacterium.

Nocardia asteroides accounts for most nocardial infections in domestic animals (Table 11.1). The most commonly encountered conditions are cutaneous and systemic infections in dogs and mastitis in dairy cattle. *Nocardia farcinica* is implicated in bovine farcy. Canine nocardiosis, due to *N. asteroides*, is acquired by inhalation, through skin wounds or by ingestion. Thoracic, cutaneous and disseminated forms of the disease are recognized. The thoracic form is characterized by fever, anorexia and respiratory distress. Sanguinopurulent fluid accumulates in the thoracic cavity. The cutaneous form presents either as an indolent ulcer or as a granulomatous swelling with discharging fistulous tracts. *Nocardia asteroides* strains show a marked variation in their susceptibility to antibiotics. A presumptive diagnosis of infection with *N. asteroides* is based on clinical findings and laboratory procedures. Smears of exudate should be stained by the Gram and modified Ziehl-Neelsen (MZN) methods. *Nocardia asteroides* is MZN-positive. When cultured aerobically on blood agar, colonies are usually visible after five days. They are white, powdery and firmly adherent to the agar.

Bovine farcy, also known as bovine nocardiosis, is limited to the tropics. Because a number of organisms, including *N. farcinica*, have been isolated from the lesions, the aetiology of the disease is uncertain. Chronic infection of superficial

Table 11.1 Disease conditions produced by *Nocardia* species in domestic animals.

Species	Hosts	Disease conditions
Nocardia asteroides	Dogs	Canine nocardiosis: — cutaneous pyogranulomas — pyogranulomatous pleural lesions and pyothorax — disseminated lesions
	Cattle	Chronic mastitis, abortion
	Pigs	Abortion
	Sheep, goats, horses	Wound infections, mastitis, pneumonia, other pyogranulomatous conditions
Nocardia farcinica	Cattle	Bovine farcy

lymphatic vessels and lymph nodes occurs. Early lesions consist of small cutaneous nodules, often on the medial aspects of the legs and on the neck. These nodules enlarge slowly and coalesce to form large swellings which rarely ulcerate. Lymphatic vessels become thickened and cord-like. Internal organs may be affected occasionally with lesions resembling tuberculosis.

Dermatophilus congolensis

This actinomycete is a Gram-positive, filamentous, branching organism. It is unusual because it produces motile zoospores about 1.5 µm in diameter. Mature zoospores produce germ tubes which develop into filaments. Within these filaments, transverse and longitudinal divisions form segments which ultimately develop into zoospores. Mature filaments may be more than 5 µm in width and contain columns of zoospores which impart a 'tram-track' appearance to the filaments. Skin infections caused by *D. congolensis* occur worldwide but are most prevalent in tropical and subtropical regions. The organism seems to persist in foci in the skin of many clinically normal animals, particularly in endemic areas. Although zoospore survival in the environment is usually limited, there may be extended survival in dry scabs.

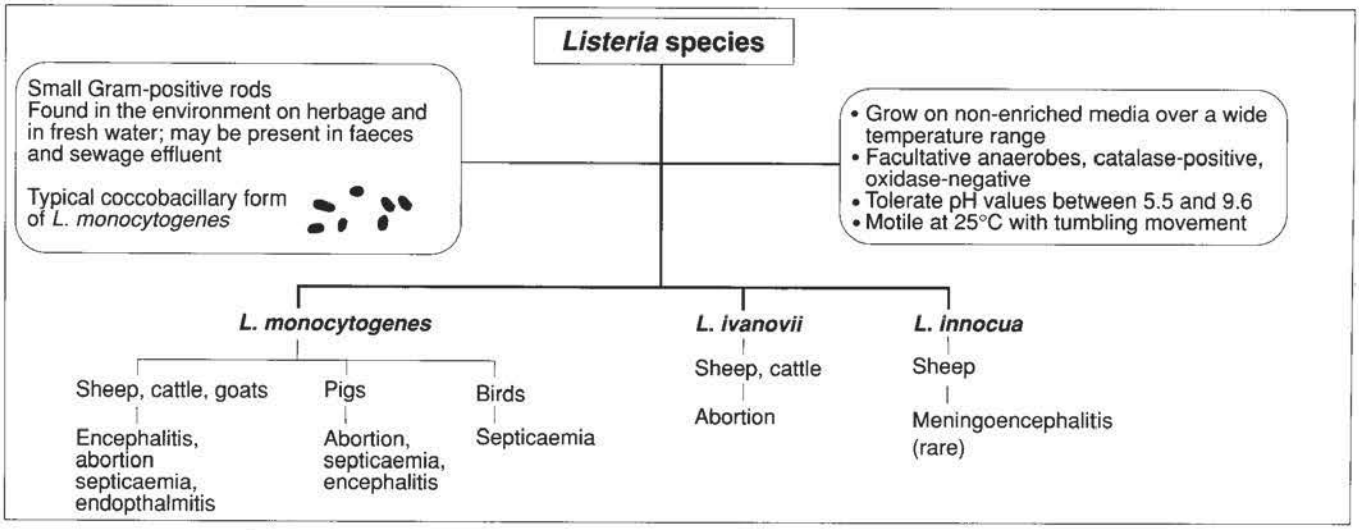
Trauma and persistent wetting predispose to skin invasion. When activated, zoospores produce germ tubes and these develop into filaments, which invade the epidermis. The ability of individual strains to invade the epidermis is related to their virulence. Keratinolytic activity may be a virulence factor. Invasion leads to an acute inflammatory response, characterized by large numbers of neutrophils which ultimately form micro-abscesses in the epidermis. Factors which depress specific immune responses, including intercurrent diseases and pregnancy, may increase host susceptibility to dermatophilosis. Infections with *D. congolensis* are usually confined to the epidermis. When skin of the lower limbs of sheep is involved, the condition is termed 'strawberry footrot'.

Although the disease affects animals of all ages, it is more prevalent and often more severe in young animals. Zoospores are most often transmitted by direct contact with infected animals. A number of blood-sucking insects may be important in disease transmission in the tropics. Economic loss derives from damage to hides and fleece.

Lesion distribution usually correlates with those areas of skin predisposed to infection. Heavy prolonged rainfall in association with warm environmental temperatures can result in lesions predominantly affecting the dorsum of farm animals. Trauma to the face and limbs of animals grazing in thorny scrub can predispose to lesions in these sites. Early lesions present as papules and are often detectable only by palpation. As lesions progress, serous exudate causes matting of hairs, resulting in a tufted appearance. Lesions may coalesce to form irregular elevated crusty scabs. Tufts of hair can be readily plucked from the lesions along with adherent scab material and underlying exudate. Scab formation tends to be more pronounced in cattle and sheep than in horses. In severe infections, lesions may be extensive and deaths may occur occasionally in calves and lambs. Diagnosis is based on clinical appearance of lesions and demonstration of *D. congolensis* in scabs. Isolation of the organisms is confirmatory.

The outcome of treatment is influenced by the severity and extent of lesions. Parenterally administered antibiotics such as long-acting oxytetracycline are usually effective. Control measures are based on minimizing the effects of predisposing factors and early treatment of clinical cases. Where feasible, grazing areas should be cleared of thorny scrub and tick infestation should be reduced by dipping or spraying with acaricides. Control of intercurrent disease reduces the severity of dermatophilosis.

12 *Listeria* species and *Erysipelothrix rhusiopathiae*



Listeria species

Most *Listeria* species are small, Gram-positive coccobacillary rods up to 2 µm in length. They are catalase-positive, oxidase-negative, motile, facultative anaerobes. The genus is composed of six species, three of which are pathogenic. *Listeria monocytogenes*, the most important of these pathogens, has been implicated worldwide in diseases of many animal species and humans. The organism can grow over a wide temperature range from 4°C to 45°C and can tolerate pH values between 5.5 and 9.5. The other two pathogens, *L. ivanovii* and *L. innocua*, are less frequently implicated in diseases of animals. *Listeria* species can replicate in the environment. They are widely distributed and can be recovered from herbage, faeces of healthy animals, sewage effluent and bodies of fresh water. Pattern of haemolysis on sheep blood agar, CAMP tests and acid production from a short range of sugars are useful differentiating laboratory methods for *Listeria* species. Colonies are small, smooth and transparent after incubation for 24 hours. Sixteen serotypes, based on cell wall and flagellar antigens, are recognized.

The clinical manifestations of infections with *Listeria* species are summarized in Table 12.1. Infection with *L. monocytogenes* usually follows ingestion of contaminated feed and may result in septicaemia, encephalitis or abortion. Organisms probably penetrate the M cells in Peyer's patches in the intestine. Spread occurs via lymph and blood to various tissues. In pregnant animals, infection results in transplacental transmission. There is evidence that the organism can invade through breaks in the oral or nasal mucosa. From this site, migration in cranial nerves is thought to be the main route of infection in neural listeriosis. Lesions in the brain stem are composed of microabscesses and perivascular lymphatic cuffs. *Listeria monocytogenes* has the ability to invade both phagocytic and non-phagocytic cells, to survive and replicate intracellularly and to transfer from cell to cell without exposure to humoral defence mechanisms.

Listeriosis in ruminants may present as encephalitis, abortion, septicaemia or endophthalmitis. Usually only one form of disease occurs in a group of affected animals. Outbreaks of listeriosis tend to be seasonal in European countries and to affect silage-fed animals in late pregnancy. *Listeria monocytogenes* can replicate in the surface layers of poor-quality silage with pH values above 5.5. The incubation period for neural listeriosis ranges from 14 to 40 days. Dullness, circling and tilting of the head are common clinical signs. Unilateral facial paralysis results in drooling of saliva and drooping of the eyelid and ear.

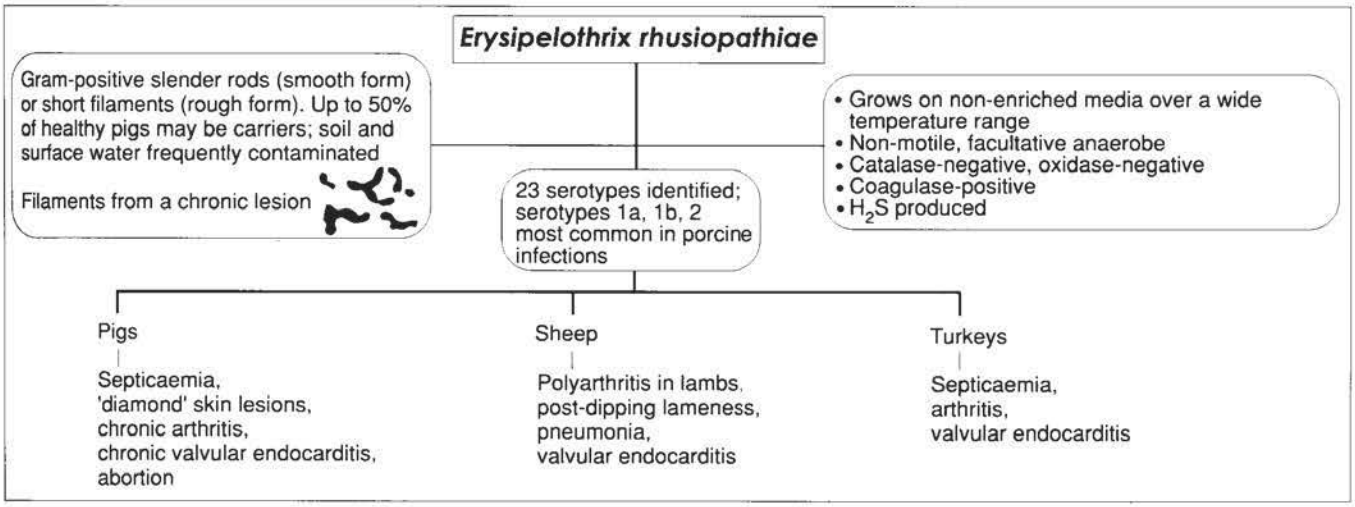
Table 12.1 Clinical manifestations of infections with *Listeria* species in domestic animals.

Species	Hosts	Forms of disease
Listeria monocytogenes	Sheep, cattle, goats	Encephalitis (neural form) Abortion Septicaemia Endophthalmitis (ocular form)
	Cattle	Mastitis (rare)
	Dogs, cats, horses	Abortion, encephalitis (rare)
	Pigs	Abortion, septicaemia, encephalitis
	Birds	Septicaemia
L. ivanovii	Sheep, cattle	Abortion
L. innocua	Sheep	Meningoencephalitis (rare)

Abortion with evidence of systemic illness may occur up to 12 days after infection. Septicaemic listeriosis, with a short incubation period, is most commonly encountered in lambs.

Characteristic neurological signs or abortion in association with silage feeding may suggest listeriosis. Appropriate specimens for laboratory examination depend on the form of the disease. Cerebrospinal fluid and tissue from the medulla and pons of animals with neurological signs should be sampled. Fresh tissue is required for isolation of the organisms and fixed tissue for histopathological examination. Specimens from cases of abortion include cotyledons, foetal abomasal contents and uterine discharges. Suitable samples from septicaemic cases include fresh liver, spleen or blood. A cold-enrichment procedure may be necessary for isolating the organism from brain tissue. Homogenized medulla, held at 4°C, is subcultured onto blood agar for 12 weeks. Identification criteria for *L. monocytogenes* include small haemolytic colonies on blood agar, a range of biochemical tests including aesculin hydrolysis, tumbling motility in broth at 25°C and a positive CAMP test with *Staphylococcus aureus*.

Response to antibiotic therapy may be poor in neural listeriosis and prolonged treatment may be required. In the early stages of septicaemic listeriosis, response to therapy is usually satisfactory. Poor-quality silage should not be fed to pregnant ruminants and if an outbreak of listeriosis is confirmed, silage feeding should be discontinued.



Erysipelothix rhusiopathiae

Erysipelothrix rhusiopathiae is a non-motile, Gram-positive, facultative anaerobe. It is catalase-negative, oxidase-negative, resistant to high salt concentrations and grows in the temperature range 5°C to 42°C and in the pH range of 6.7 to 9.2. Isolates from animals with acute infections form smooth colonies while isolates from chronically infected animals form rough colonies. Smears from smooth colonies yield slender rods whereas rough colonies are usually composed of short filaments which decolourize readily. The bacterium grows on nutrient agar but growth is improved in media containing blood or serum.

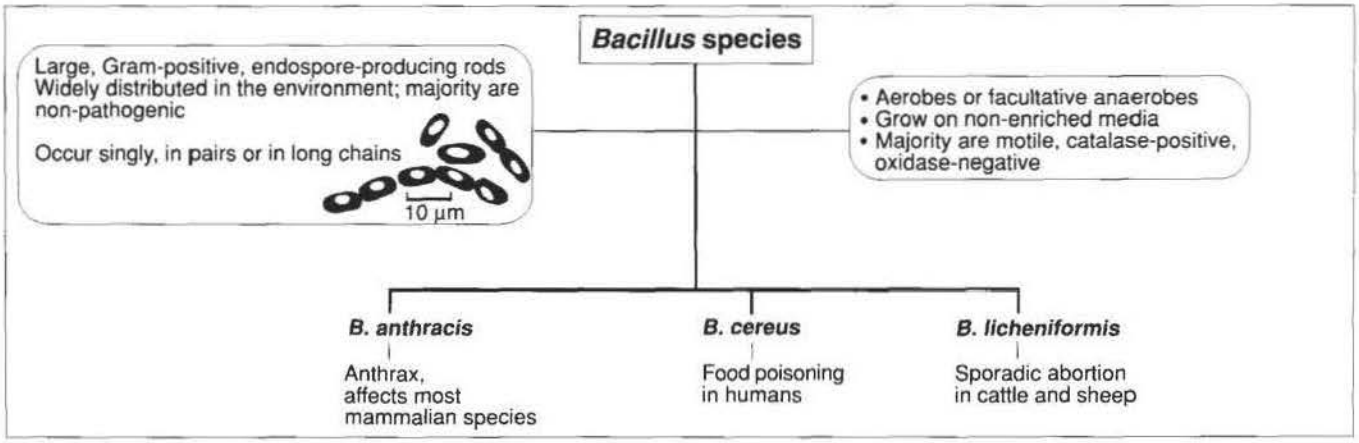
Erysipelothrix rhusiopathiae causes erysipelas in pigs and turkeys worldwide. Sheep and other domestic animals are occasionally infected. Up to 50% of healthy pigs may harbour *E. rhusiopathiae* in their tonsils. Carrier pigs may excrete the organism in faeces and in oronasal secretions.

Infection is usually acquired by ingestion of material contaminated with pig faeces. Entry may occur through the tonsils, skin or mucous membranes. Virulence factors include a capsule which protects the organism against phagocytosis and an enzyme which may enhance cell penetration. Swine erysipelas can occur in four forms. The septicaemic and cutaneous ('diamond') forms are acute, while arthritis and vegetative endocarditis are chronic forms of the disease. Chronic arthritis has the most significant negative impact on productivity. Septicaemia occurs after an incubation period of about three days. Some pigs may be found dead; others are febrile and depressed. Mortality may be high in some outbreaks and pregnant sows may abort. In the diamond-skin form, systemic signs are less severe. Pigs are febrile and cutaneous lesions progress from small, light pink or purple, raised areas to more extensive diamond-shaped erythematous plaques. Arthritis, which is commonly encountered in older pigs, can present as stiffness, lameness or reluctance to bear weight on affected limbs. Joint lesions can progress to erosion of articular cartilage with eventual fibrosis and ankylosis. In vegetative endocarditis, the least common form, wart-like thrombotic masses are present usually on the mitral valves. Many affected animals are asymptomatic but some may develop congestive heart failure or die suddenly if stressed by physical exertion or by pregnancy. Clinical presentation and the type and location of lesions may suggest swine erysipelas. Diamond-shaped lesions are pathognomonic. Live and attenuated vaccines are available for the prevention of erysipelas in pigs.

Turkey erysipelas affects birds of all ages. Toms may excrete the organisms in their semen and turkey hens may die suddenly within five days of artificial insemination.

Non-suppurative polyarthritis of lambs may result from entry of *E. rhusiopathiae* through the navel or, more commonly, through docking or castration wounds. Post-dipping lameness, which affects older lambs and adult sheep is due to cellulitis and laminitis.

13 *Bacillus* species



Most *Bacillus* species are large, Gram-positive, endospore-producing rods up to 10 µm in length. In smears from tissues or cultures, cells occur singly, in pairs or in long chains. The genus is comprised of more than 50 species with diverse characteristics. *Bacillus* species are catalase-positive, aerobic or facultatively anaerobic and, with the exception of *Bacillus anthracis* and *B. mycoides*, motile. Most species are saprophytes with no pathogenic potential. *Bacillus anthracis* is the most important pathogen in the group.

Bacillus species are widely distributed in the environment mainly because they produce highly resistant endospores. In soil, endospores of *B. anthracis* can survive for more than 50 years. The ability to grow aerobically and to produce catalase distinguishes *Bacillus* species from clostridia, which are also Gram-positive, endospore-forming rods. Differentiation of *Bacillus* species is largely based on colonial characteristics and biochemical tests.

The major disease conditions caused by bacteria in this group are listed in Table 13.1. Anthrax is the most important of these diseases. *Bacillus licheniformis* is an emerging pathogen in the group as a cause of abortion in sheep and cattle. Because they have some similar characteristics, *B. anthracis* and *B. cereus* require careful differentiation (Table 13.2).

Anthrax

Anthrax is a severe disease which affects virtually all mammalian species including humans. The disease, which occurs worldwide, is endemic in some countries. Ruminants are highly susceptible, often developing a rapidly fatal septicaemic form of the disease. Pigs and horses are moderately susceptible to infection, while carnivores are comparatively resistant. Birds are almost totally resistant to infection.

Endospore formation is the most important factor in the persistence and spread of anthrax. Outbreaks of disease in herbivores can occur when pastures are contaminated by spores originating from buried carcases. Spores may be brought to the surface by flooding, excavation, subsidence, or by the activity of earthworms. Sporadic outbreaks of disease have been associated with the importation of contaminated meat-and-bone meal, fertilizers of animal origin and hides. Infection is usually acquired by ingestion of spores and, less commonly, by inhalation or through skin abrasions.

The virulence of *B. anthracis* derives from the presence of a capsule and the ability to produce a complex toxin. Both virulence factors are encoded by plasmids and are required for disease production. The capsule, composed of poly-D-glutamic acid, inhibits phagocytosis. The complex toxin consists of three antigenic components: protective antigen,

Table 13.1 Clinical manifestations of diseases caused by *Bacillus anthracis* and other *Bacillus* species.

Bacillus species	Susceptible species	Clinical manifestations
B. anthracis	Cattle, sheep	Fatal peracute or acute septicaemic anthrax
	Pigs	Subacute anthrax with oedematous swelling in pharyngeal region; an intestinal form with higher mortality is less common
	Horses	Subacute anthrax with localized oedema; septicaemia with colic and enteritis sometimes occurs
	Humans	Skin, pulmonary and intestinal forms of anthrax are recorded in humans periodically
B. cereus	Cattle	Mastitis (rare)
	Humans	Food poisoning, eye infections
B. licheniformis	Cattle, sheep	Sporadic abortion

Table 13.2 Differentiating features of *Bacillus anthracis* and *B. cereus*.

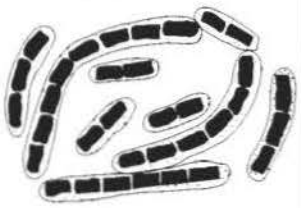
Feature	*B. anthracis*	*B. cereus*
Motility	Non-motile	Motile
Appearance on sheep blood agar	Non-haemolytic	Haemolytic
Susceptibility to penicillin (10 unit disc)	Susceptible	Resistant
Lecithinase activity on egg yolk agar	Weak and slow	Strong and rapid
Effect of gamma phage	Lysis	Lysis rare
Pathogenicity for animals (application to scarified area at tail base of mouse)	Death in 24 to 48 hours	No effect

oedema factor and lethal factor. In naturally-occurring disease, local effects of the complex toxin include swelling and darkening of tissues due to oedema and necrosis. When septicaemia occurs, increased vascular permeability and extensive haemorrhage lead to shock and death.

The incubation period of anthrax ranges from hours to days. In cattle and sheep, the disease is usually septicaemic and rapidly fatal. Although most animals are found dead without premonitory signs, pyrexia, with temperatures up to 42°C, depression, congested mucosae and petechiae may be observed antemortem. In cattle, postmortem findings include rapid bloating, incomplete rigor mortis, widespread ecchymotic haemorrhages and oedema, dark unclotted blood and blood-stained fluid in body cavities. An extremely large soft spleen is characteristic of the disease in cattle. Splenomegaly and oedema are less prominent postmortem features in affected sheep. In pigs, infection generally results in oedematous swelling of the throat and head along with regional lymphadenitis. Some affected pigs may survive.

Chains of *Bacillus anthracis* as they appear in a thin blood smear stained with polychrome methylene blue

The blue-staining, square-ended organisms are surrounded by pink capsules

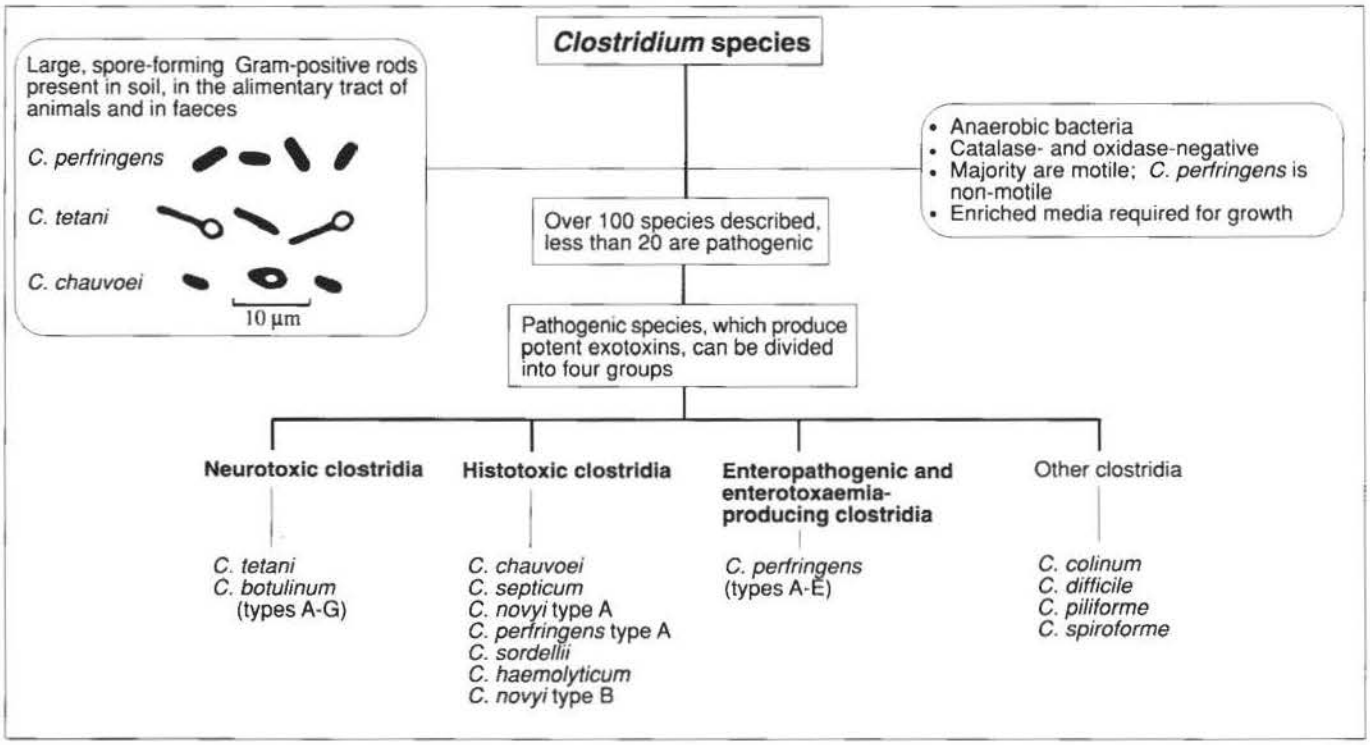


Carcases of animals which have died from anthrax are bloated, putrefy rapidly and do not exhibit rigor mortis. Dark, unclotted blood may issue from the mouth, nostrils and anus. The carcases of such animals should not be opened because this will facilitate sporulation with the risk of long-term environmental contamination. Peripheral blood from the tail vein of ruminants or peritoneal fluid from pigs should be collected into a sterile syringe. Thin smears of blood or fluid, stained with polychrome methylene blue, reveals chains of square-ended, blue-staining rods surrounded by a pink capsule (M'Fadyean reaction). Blood and MacConkey agars are inoculated with the suspect specimens and incubated aerobically at 37°C for 24 to 48 hours. Identification criteria for isolates include colonial morphology, microscopic appearance in Gram-stained smears, absence of growth on MacConkey agar and other test procedures (Table 13.2). New molecular diagnostic methods, based on the use of PCR to amplify specific virulence-related plasmid markers are being developed.

If administered early in the course of the disease, high doses of penicillin G or oxytetracycline may prove effective. Suspect cases of anthrax must be reported immediately to appropriate regulatory authorities. In endemic regions, annual vaccination of sheep and cattle with the Sterne strain spore vaccine is advisable. In non-endemic regions, movement of animals, feed and bedding must be prohibited following a disease outbreak. Personnel implementing control measures should wear protective clothing and footwear. Carcases should be incinerated or buried deeply away from water courses. Contaminated equipment should be disinfected with 10% formalin. In-contact animals should be isolated and kept under close observation for at least two weeks. Contaminated buildings should be sealed and fumigated with formaldehyde before removal of bedding. Following removal of fittings and bedding, the building should be sprayed with 5% formalin which should be left to act for 10 hours before final washing.

Three main forms of anthrax occur in humans. Cutaneous anthrax (malignant pustule) is the result of endospores entering abraded skin. If not treated promptly, this localized lesion can progress to septicaemia. Pulmonary anthrax ('wool-sorters' disease') follows inhalation of spores, while intestinal anthrax results from the ingestion of infective material. The disease may prove fatal in the absence of early treatment.

14 *Clostridium* species 1



Clostridia are saprophytes which are found in soil, fresh water and marine sediments. They constitute part of the normal intestinal flora and some may be sequestered as endospores in muscle or liver. Enriched blood agar is suitable for the culture of clostridia. Anaerobic jars containing hydrogen supplemented with 5% to 10% carbon dioxide provide a suitable atmosphere for growth.

Differentiation of clostridia

Clostridia can be differentiated by their colonial morphology, biochemical tests and by toxin neutralization methods. Specific toxins in body fluids or intestinal contents can be identified by toxin neutralization or protection tests in laboratory animals, usually mice. Immunoassay methods such as ELISA can also be used for toxin detection and these tests have replaced many mouse bioassay tests. The presence of histotoxic clostridia in lesions can be demonstrated rapidly by fluorescent antibody techniques.

Neurotoxic clostridia

The neurotoxic clostridia, *C. tetani* and *C. botulinum*, elaborate potent neurotoxins. The neurotoxin of *C. tetani* is produced by organisms replicating locally in damaged tissues. When absorbed, toxin exerts its effect on synaptic junctions remote from the site of toxin production. The neurotoxin of *C. botulinum* is usually produced by bacteria replicating in organic matter or in the anaerobic conditions in contaminated cans of meat or vegetables. When absorbed from the gastrointestinal tract into the bloodstream, the toxin affects the functioning of neuromuscular junctions.

Tetanus

This acute and potentially fatal intoxication is caused by the toxin of *C. tetani* which affects many species including humans. Species susceptibility to toxin varies: horses and humans are highly susceptible, ruminants and pigs are moderately susceptible while poultry are resistant.

Infection occurs when endospores of *C. tetani* from soil or faeces are introduced into damaged tissue. Contamination of deep wounds in unvaccinated horses or ruminants increases the possibility of tetanus developing. The presence of necrotic tissue or contaminating facultative anaerobes may create the anaerobic conditions in a wound in which *C. tetani* spores can germinate. Vegetative bacteria multiplying in necrotic tissue produce the potent neurotoxin, tetanospasmin, which is responsible for the clinical signs of tetanus. Although ten serological types of *C. tetani* can be distinguished by their flagellar antigens, the neurotoxin produced is antigenically uniform and antibodies induced by one neurotoxin neutralize the neurotoxins produced by others. The neurotoxin binds irreversibly to ganglioside receptors. Toxin is transferred trans-synaptically to its site of action in the terminals of inhibitory neurons where it blocks pre-synaptic transmission of inhibitory signals. Because release of inhibitory neurotransmitters is prevented, spastic

paralysis results. Bound toxin is not neutralized by antitoxin.

The incubation period of tetanus may be up to 10 days but can extend to three weeks. Clinical effects of the neurotoxin, which are similar in all domestic animals, include stiffness, localized spasms, altered heart and respiratory rates, dysphagia and altered facial expression. Mild tactile or auditory stimuli may precipitate tonic contraction of muscles. Spasm of masticatory muscles may lead to 'lockjaw'. In horses, generalized muscle stiffness can result in a 'saw-horse' stance. Animals which recover from tetanus are not necessarily immune, as the low dose of toxin capable of producing disease may not induce neutralizing antibodies. Diagnosis is based on clinical signs, a history of recent trauma, Gram-stained smears from lesions and anaerobic culture of *C. tetani* from wound tissue. Serum from affected animals may be used to demonstrate neurotoxin using mouse inoculation.

Treatment procedures include prompt antitoxin administration, large doses of penicillin to inhibit growth of *C. tetani* in lesions and surgical debridement of wounds. Prevention of tetanus in farm animals is based on routine vaccination with tetanus toxoid, followed by booster doses at specified intervals.

Botulism

This potentially fatal intoxication is usually acquired by ingestion of pre-formed toxin. The endospores of *C. botulinum* are distributed in soils and aquatic environments worldwide. Eight types of *C. botulinum* are recognized on the basis of toxins (A, B, C_{α}, C_{β}, D, E, F, G) which they produce. The usual sources of toxins of *C. botulinum* types A to G for susceptible species are summarized in Table 14.1. *Clostridium botulinum* types C and D cause most outbreaks of botulism in domestic animals. Outbreaks of disease occur most commonly in waterfowl, cattle, horses, sheep, mink, poultry and farmed fish. Botulism in cattle has been associated with ingestion of poultry carcases present in ensiled poultry litter used as bedding or spread on pasture. Waterfowl and other birds can acquire toxin from dead invertebrates, decaying vegetation or from the consumption of maggots containing toxin.

The neurotoxins of *C. botulinum* are the most potent biological toxins known. When absorbed from the gastrointestinal tract, preformed toxin acts at the neuromuscular junctions of cholinergic nerves and at peripheral autonomic synapses. Hydrolysis of synaptobrevins causes irreversible interference with the release of the transmitter, acetylcholine, resulting in flaccid paralysis. Death results from paralysis of respiratory muscles.

The clinical signs of botulism, which develop within days after toxin ingestion, are similar in all species. Dilated pupils, dry mucous membranes, decreased salivation, tongue flaccidity and dysphagia are features of the disease in farm animals.

Table 14.1 Toxins of *Clostridium botulinum*.

Toxin	Source	Susceptible species
Type A	Meat, canned products	Humans
	Toxico-infection	Infants
	Meat, carcases	Mink, dogs, pigs
Type B	Meat, canned products	Humans
	Toxico-infection	Infants
	Toxico-infection	Foals (up to two months of age)
Type C	Dead invertebrates, maggots, rotting vegetation and carcases of poultry	Waterfowl, poultry
	Ensiled poultry litter, baled silage (poor quality), hay or silage contaminated with rodent carcases	Cattle, sheep, horses
	Meat, especially chicken carcases	Dogs, mink, lions, monkeys
Type D	Carcases, bones	Cattle, sheep
	Feed contaminated with carcases	Horses
Type E	Dead invertebrates, sludge in earth-bottomed ponds	Farmed fish
	Fish	Fish-eating birds, humans
Type F	Meat, fish	Humans
Type G	Soil-contaminated food	Humans (in Argentina)

Incoordination and knuckling of the fetlocks is followed by flaccid paralysis and recumbency. Death may follow within days. In birds, there is progressive flaccid paralysis which initially affects legs and wings. Paralysis of neck muscles ('limberneck') is evident only in long-necked species.

Clinical signs and the history may suggest botulism. Confirmation requires the demonstration of toxin in the serum of affected animals by mouse inoculation, or by immunological methods such as ELISA. Toxin neutralization tests in mice, using monovalent antitoxins, can be used to identify the specific toxins involved.

Mildly affected animals may recover slowly without therapy. Polyvalent antiserum is effective in neutralizing unbound toxin but cost and availability limit this treatment. Vaccination of cattle with toxoid may be necessary in endemic regions in South Africa and Australia. Routine vaccination of farmed mink and foxes may be advisable.

15 *Clostridium* species 2

The histotoxic clostridia, through exotoxin production, cause both local tissue necrosis and systemic effects which may be lethal. Clostridia, which produce enterotoxaemia and enteropathy, replicate in the intestinal tract and elaborate toxins which produce both localized and generalized tissue damage.

Histotoxic clostridia

Endospores of histotoxic clostridia are widely distributed in soil. Histotoxic clostridia and the diseases which they produce are presented in Table 15.1. Although it is probable that the majority of ingested endospores are excreted in the faeces, some may be transported to the tissues in phagocytyes. Tissue injury leading to reduced oxygen tension is required for spore germination. Endogenous infections which include blackleg, infectious necrotic hepatitis and bacillary haemoglobinuria, result from the activation of dormant spores in muscle or liver. The exogenous infections, malignant oedema and gas gangrene, result from the introduction of clostridial organisms into wounds.

The clinical infections produced by histotoxic clostridia include blackleg, malignant oedema, gas gangrene, braxy, infectious necrotic hepatitis and bacillary haemoglobinuria.

Blackleg, an acute disease of cattle and sheep caused by *C. chauvoei*, which is usually endogenous, occurs worldwide. The disease occurs in young thriving cattle from three months to two years of age. Latent spores in muscle become activated through traumatic injury. The large muscle masses of the limbs, back and neck are frequently affected. Skeletal muscle damage is manifest by lameness, swelling and crepitation due to gas accumulation.

Malignant oedema and gas gangrene are exogenous, necrotizing, soft tissue infections. *Clostridium septicum* is often associated with malignant oedema and *C. perfringens* type A with gas gangrene. Malignant oedema manifests as cellulitis with minimal gangrene and gas formation. In gas gangrene, extensive bacterial invasion of damaged muscle tissue occurs. Gas production is detectable clinically as subcutaneous crepitation.

Braxy is an abomasitis of sheep caused by the exotoxins of *C. septicum*. The disease occurs in winter during periods of heavy frost or snow. Ingestion of frozen herbage may cause devitalization of abomasal tissue at its point of contact with the rumen, allowing invasion by *C. septicum*.

Infectious necrotic hepatitis (black disease) is an acute

Table 15.1 Histotoxic clostridia, their major toxins and the diseases produced in domestic animals.

Clostridium species	Disease	Toxin	
		Name	Biological activity
C. chauvoei	Blackleg in cattle and sheep	α	Lethal, haemolytic, necrotizing
		β	Deoxyribonuclease
		γ	Hyaluronidase
		δ	Oxygen-labile haemolysin
C. septicum	Malignant oedema in cattle, pigs and sheep Abomasitis in sheep (braxy) and occasionally in calves	α	Lethal, haemolytic, necrotizing
		β	Deoxyribonuclease
		γ	Hyaluronidase
		δ	Oxygen-labile haemolysin
C. novyi type A	'Big head' in young rams Wound infections	α	Necrotizing, lethal
C. perfringens type A	Necrotic enteritis in chickens Necrotizing enterocolitis in pigs Gas gangrene	α	Haemolytic, necrotizing, lethal, lecithinase
C. sordellii	Myositis in cattle, sheep and horses	α	Lecithinase
	Abomasitis in lambs	β	Oedema-producing lethal factor
C. novyi type B	Infectious necrotic hepatitis (black disease) in sheep and occasionally in cattle	α	Necrotizing, lethal
		β	Necrotizing, haemolytic, lethal, lecithinase
C. haemolyticum	Bacillary haemoglobinuria in cattle and occasionally in sheep	β	Necrotizing, haemolytic, lethal, lecithinase

Table 15.2 Types of *Clostridium perfringens*, their major toxins and associated diseases.

Clostridium perfringens	Disease	Toxin	
		Name	Biological activity
Type A	Necrotic enteritis in chickens Necrotizing enterocolitis in pigs	α (significant toxin)	Lecithinase
	Canine haemorrhagic gastroenteritis	Enterotoxin	Cytotoxic
Type B	Lamb dysentery Haemorrhagic enteritis in calves and foals	α β (significant toxin) ε (exists as a prototoxin and requires activation by proteolytic enzymes)	Lecithinase Lethal, necrotizing Increases intestinal and capillary permeability, lethal
Type C	'Struck' in adult sheep Sudden death in goats and feedlot cattle Necrotic enteritis in chickens Haemorrhagic enteritis in neonatal piglets	α β (significant toxin) Enterotoxin	Lecithinase Lethal, necrotizing Cytotoxic
Type D	Pulpy kidney in sheep Enterotoxaemia in calves, adult goats and kids	α ε (significant toxin, exists as a prototoxin and requires activation by proteolytic enzymes)	Lecithinase Increases intestinal and capillary permeability, lethal
Type E	Haemorrhagic enteritis in calves Enteritis in rabbits	α ι (significant toxin)	Lecithinase Lethal

disease affecting sheep and occasionally cattle. Hepatic necrosis is caused by exotoxins of *C. novyi* type B replicating in liver tissue which has been damaged by immature *Fasciola hepatica* or other migrating parasites.

Bacillary haemoglobinuria, which occurs primarily in cattle, is an endogenous infection with *C. haemolyticum*. The clostridial endospores remain dormant in the liver, probably in Kupffer cells. Fluke migration facilitates spore germination and the vegetative cells produce β toxin, a lecithinase, which causes intravascular haemolysis in addition to hepatic necrosis. Haemoglobinuria is a major clinical feature of the disease.

Fluorescent antibody techniques are used extensively for the diagnosis of diseases caused by histotoxic clostridia. *Clostridium perfringens* is cultured anaerobically on blood agar at 37°C for 48 hours. The Nagler reaction, a plate neutralization test, identifies the α toxin of *C. perfringens* which has lecithinase activity. A PCR-based method for the identification of *C. chauvoei* has been described.

Enteropathogenic clostridia

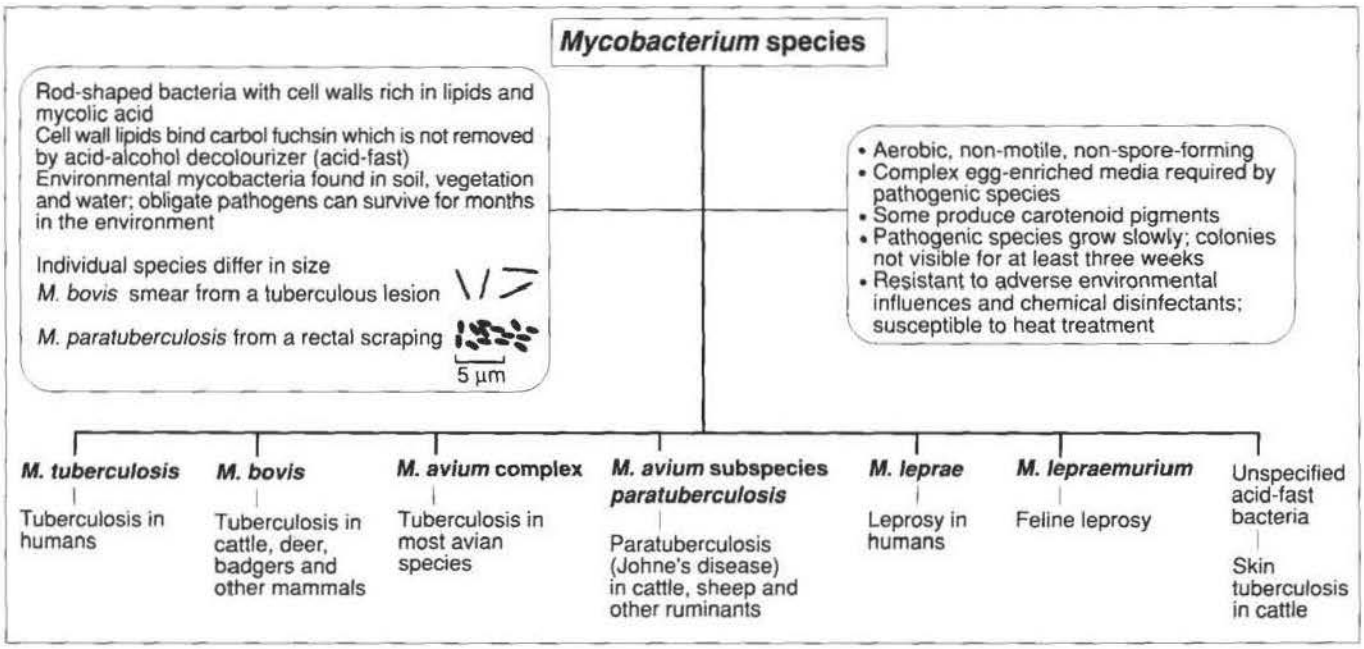
Clostridium perfringens types A to E produce a number of potent, immunologically distinct exotoxins which cause the local and systemic effects encountered in enterotoxaemias. The toxins produced by *C. perfringens* types A to E, their biological activities and associated diseases are presented in Table 15.2.

Lamb dysentery, caused by *C. perfringens* type B, can cause high mortality in lambs during the first week of life. Many animals die suddenly and the high susceptibility of this group is attributed to the absence of microbial competition and the low proteolytic activity in the neonatal intestine. Infection with *C. perfringens* type C causes 'struck', an acute enterotoxaemia in adult sheep in defined geograhical regions. The disease, which occurs in sheep at pasture, usually manifests as sudden death.

Pulpy kidney disease, caused by *C. perfringens* type D, occurs in sheep worldwide. Ingestion of excessive quantities of food may lead to the transfer of partially digested food from the rumen into the intestine and its high starch content is a suitable substrate for rapid clostridial proliferation. The ε toxin, which exists as a prototoxin and requires activation by proteolytic enzymes, produces toxaemia and death shortly after clinical signs emerge. Focal symmetrical encephalomalacia, a manifestation of the subacute effects of the ε toxin on the vasculature, is characterized by symmetrical haemorrhagic lesions in the basal ganglia and midbrain.

Direct smears from the mucosa or contents of the small intestine of recently dead animals which contain substantial numbers of large Gram-positive rods are consistent with enterotoxaemia. Toxin neutralization tests using mouse and guinea-pig inoculation can definitively identify the toxins of *C. perfringens* present in the contents of recently dead animals. ELISA can be used as an alternative to *in vivo* assays for demonstrating toxin in intestinal contents. Vaccination is the principal control method. Ewes should be vaccinated with toxoid six weeks before lambing to ensure passive protection for lambs. Sudden dietary changes and other factors predisposing to enterotoxaemias should be avoided.

16 *Mycobacterium* species 1



Mycobacteria are aerobic, non-spore-forming, non-motile, rod-shaped acid-fast bacilli. Individual species differ in size; the rods of *Mycobacterium bovis* and *M. avium* subspecies *avium* are slender and up to 4 µm in length, whereas those of *M. avium* subspecies *paratuberculosis* are broad and are usually less than 2 µm long. Although mycobacteria are cytochemically Gram-positive, the high lipid and mycolic acid content of cell walls prevents uptake of the dyes employed in the Gram stain. The cell wall lipids bind carbol fuchsin which is not removed by the Ziehl-Neelsen (ZN) staining method. Bacilli which stain red by this method are called acid-fast or ZN-positive.

The mycobacteria include diverse species ranging from environmental saprophytes and opportunistic invaders to obligate pathogens. Mycobacterial diseases in domestic animals are usually chronic and progressive. The closely-related members of the *M. tuberculosis* complex (*M.tuberculosis*, *M. bovis* and *M. africanum*) cause tuberculosis in humans. Environmental mycobacteria are found in soil, on vegetation and in water. Obligate pathogens, shed by infected animals, can survive in the environment for extended periods.

The ZN staining method is used to differentiate mycobacteria from other bacteria. Differentation of pathogenic mycobacteria relies on cultural characteristics, biochemical tests, animal inoculation, chromatographic analysis and molecular techniques. Strict safety precautions must be observed when working with material containing mycobacteria. Pathogenic mycobacteria grow slowly and colonies are not evident until cultures have been incubated for at least three weeks. In contrast, colonies of rapidly growing saprophytes are visible within days. Pathogenic species of mycobacteria can be distinguished by their colonial appearance on egg-based media, the influence of added glycerol and sodium pyruvate on growth rate and pigment production. Guinea-pig and rabbit inoculation was used in the past to differentiate *M. tuberculosis* from *M. bovis* and *M. avium*. Molecular techniques which include DNA probes, nucleic acid amplification procedures and DNA restriction endonuclease analyses are being developed as sensitive and rapid methods for the detection of mycobacteria in tissue samples. Methods used for differentiating important pathogenic mycobacteria are presented in Table 16.1.

The diseases caused by pathogenic mycobacteria are presented in Table 16.2. The major pathogenic *Mycobacterium* species which affect domestic animals exhibit a considerable degree of host specificity although they can produce sporadic disease in a number of other hosts. Diseases in domestic animals caused by mycobacteria include tuberculosis in avian and mammalian species, paratuberculosis in ruminants and feline leprosy. Two other clinical conditions, skin tuberculosis and bovine farcy are associated with the presence of acid-fast bacteria in lesions. Avian tuberculosis, which occurs worldwide, is usually caused by members of the *M. avium* complex. The disease is encountered most often in free-range adult birds and transmission is usually by the faecal-oral route. Non-specific clinical signs including dullness, emaciation and lameness develop in affected birds only when the disease is at an advanced stage. At postmortem examination, granulomatous lesions are characteristically present in the liver, spleen, bone marrow and intestines. Diagnosis is based on postmortem findings and on the demonstration of large numbers of ZN-positive bacilli in smears from lesions.

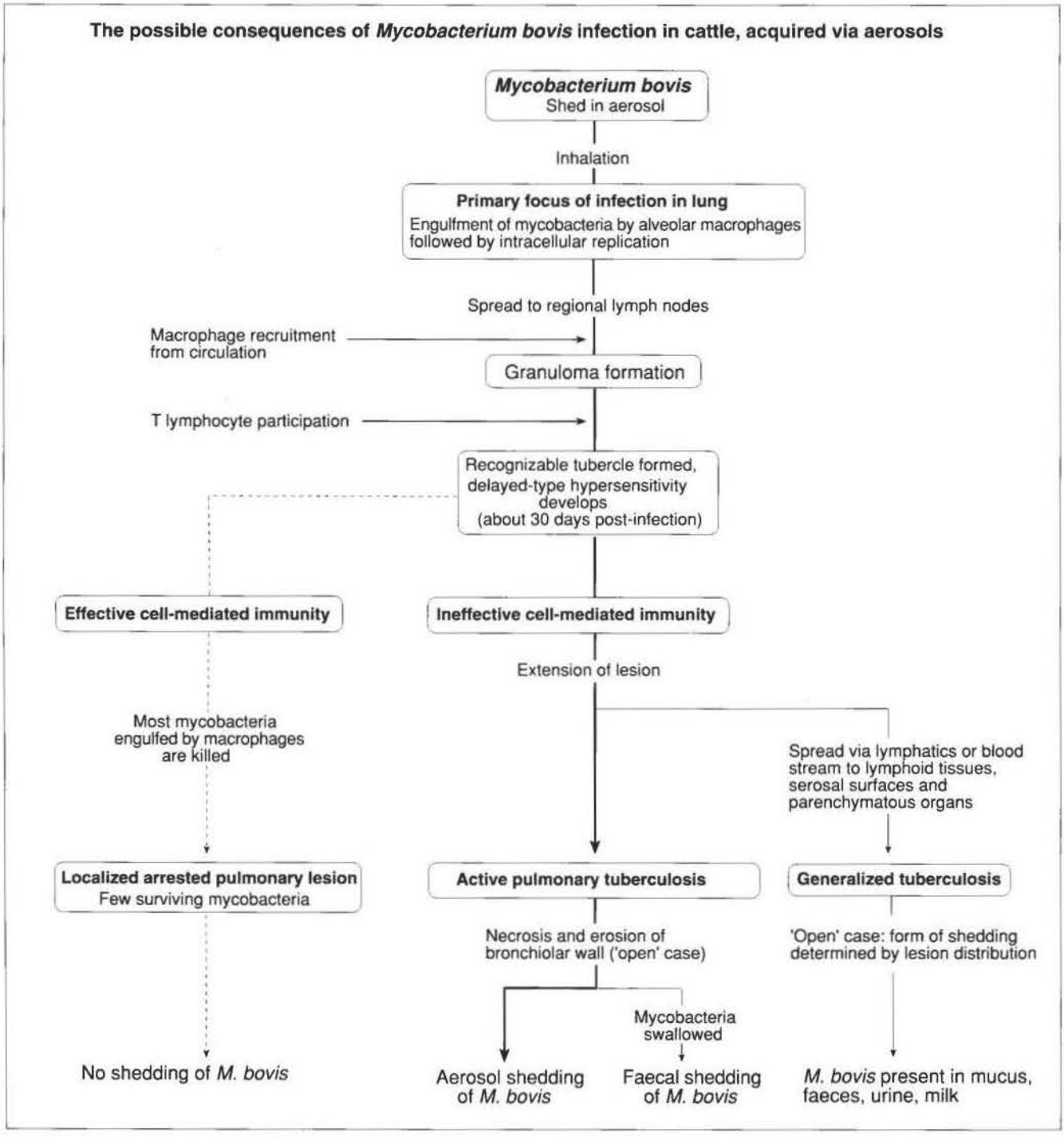
Table 16.1 Clinical significance, growth characteristics and biochemical differentiation of pathogenic mycobacteria.

	M. tuberculosis	*M. bovis*	*M. avium* complex	*M. avium* subsp. *paratuberculosis*
Significance of infection	Important in humans and occasionally in dogs	Important in cattle and occasionally in other domestic animals and humans	Important in free-range domestic poultry, opportunistic infections in humans and domestic animals	Important in cattle and other ruminants
Cultural characteristics and requirements				
Growth rate	Slow (3-8 weeks)	Slow (3-8 weeks)	Slow (2-6 weeks)	Very slow (up to 16 weeks)
Optimal incubation temperature	37°C	37°C	37-43°C	37°C
Atmospheric requirements	Aerobic	Aerobic	Aerobic	Aerobic
Colonial features	Rough, buff, difficult to break apart	Cream-coloured, raised with central roughness, break apart easily	Sticky, off-white, break apart easily	Small, hemispherical; some pigmented
Essential growth supplement	None	None	None	Mycobactin
Effect of added glycerol	Enhanced growth (eugonic)	Growth inhibited (dysgonic)	Enhanced growth (eugonic)	
Effect of added sodium pyruvate	No effect	Enhanced growth	No effect	

Table 16.2 Mycobacteria which are pathogenic for animals and humans.

***Mycobacterium* species**	**Main hosts**	**Species occasionally infected**	**Disease**
M. tuberculosis	Humans, captive primates	Dogs, cattle, psittacine birds, canaries	Tuberculosis (worldwide)
M. bovis	Cattle	Deer, badgers, possums, humans, cats, other mammalian species	Tuberculosis
M. africanum	Humans		Tuberculosis (regions in Africa)
M. avium complex	Most avian species except psittacines	Pigs, cattle	Tuberculosis
M. microti	Voles	Rarely other mammalian species	Tuberculosis
M. marinum	Fish	Humans, aquatic mammals, amphibians	Tuberculosis
M. leprae	Humans	Armadillos, chimpanzees	Leprosy
M. lepraemurium	Rats, mice	Cats	Rat leprosy, feline leprosy
M. avium subsp. *paratuberculosis*	Cattle, sheep, goats, deer	Other ruminants	Paratuberculosis (Johne's disease)
Unspecified acid-fast bacteria	Cattle		Associated with skin tuberculosis
M. senegalense, *M. farcinogenes*	Cattle		Implicated in bovine farcy

17 *Mycobacterium* species 2



Tuberculosis in cattle

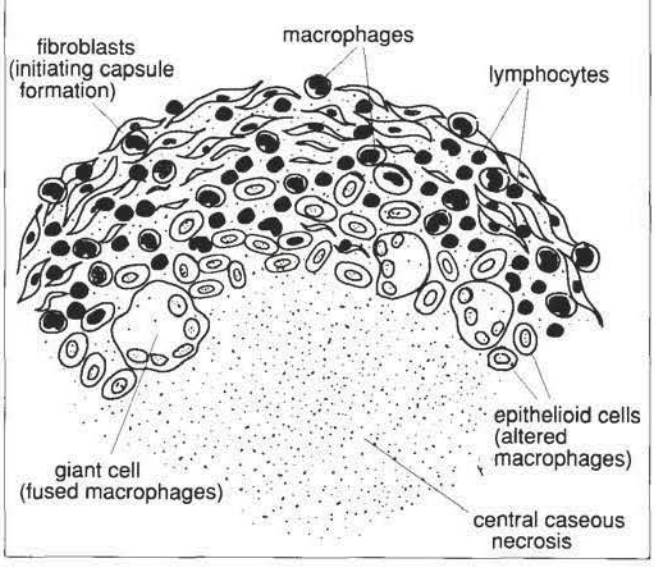
Bovine tuberculosis, caused by *M. bovis*, occurs worldwide. Because of the zoonotic implications of the disease and production losses due to its chronic progressive nature, eradication programmes have been introduced in many countries. Although *M. bovis* can survive for several months in the environment, transmission is mainly through aerosols generated by infected cattle. Dairy cattle in particular are at risk because husbandry methods allow close contact between animals at milking and when housed during winter months. Wildlife reservoirs of *M. bovis* are major sources of infection for grazing cattle in some countries. They include the badger in Europe, the brush-

tailed possum in New Zealand and the Cape buffalo and other ruminants in Africa. Deer are particularly susceptible and may act as reservoirs of infection for cattle.

The virulence of *M. bovis* relates to its ability to survive and multiply in host macrophages. Survival within the cytoplasm of macrophages is promoted by interference with phagosome-lysosome fusion and failure of lysosomal digestion. Clinical signs are evident only in advanced disease and cattle with extensive lesions can appear to be in good health. Loss of condition may become evident as the disease progresses. Tuberculous mastitis facilitates spread of infection to calves and cats, and is of major public health importance.

In the early stages of disease, lesions may be difficult to detect at postmortem examination. In older lesions, fibroplasia produces early capsule formation and there is central caseous necrosis, detectable grossly as yellowish cheesy material. The tuberculin test, based on a delayed-type hypersensitivity to mycobacterial tuberculoprotein, is the standard antemortem test in cattle. Reactivity in cattle is usually detectable 30 to 50 days after infection. Tuberculin, prepared from mycobacteria and called purified protein derivative (PPD) is injected intradermally to detect sensitization. In the single intradermal (caudal fold) test, 0.1 ml of bovine PPD is injected into the caudal fold of the tail and the injection site is examined 72 hours later. A positive reaction is characterized by a hard or oedematous swelling. In the comparative intradermal test, 0.1 ml of avian PPD and 0.1 ml of bovine PPD are injected intradermally into separate clipped sites on the side of the neck about 12 cm apart. Skin thickness at the injection sites is measured with calipers before injection of tuberculins and after 72 hours. An increase in skin thickness at the injection site of bovine PPD which exceeds that at the avian PPD injection site by 4 mm or more is interpreted as evidence of infection and the animal is termed a reactor. False positive reactions may be attributed to sensitization to mycobacteria other than *M. bovis*. False negative results may be recorded if cattle are tested before delayed-type hypersensitivity to tuberculoproteins develops. In some cattle an unresponsive state, referred to as anergy, may accompany advanced tuberculosis. Cows may be unresponsive to the tuberculin test during the early postpartum period. Blood-based tests which are being evaluated include gamma interferon assay, ELISA for detecting circulating antibodies and lymphocyte transformation. Specimens suitable for laboratory demonstration or culture of *M. bovis* include lymph nodes, tissue lesions, aspirates and milk. Decontamination of specimens is required, to eliminate fast-growing contaminating bacteria, before slants of Lowenstein-Jensen medium containing 0.4% sodium pyruvate are inoculated. The slants are cultured aerobically for up to 8 weeks. Identification criteria for isolates include growth rate, positive ZN-staining of bacilli in smears from colonies, biochemical profile and analytical and molecular techniques. Tuberculin testing, followed by isolation and slaughter of reactors is the basis of national eradication schemes in many countries. Routine meat inspection forms part of the surveillance programme for bovine tuberculosis in many countries.

Microscopic appearance of a bovine tuberculous lesion

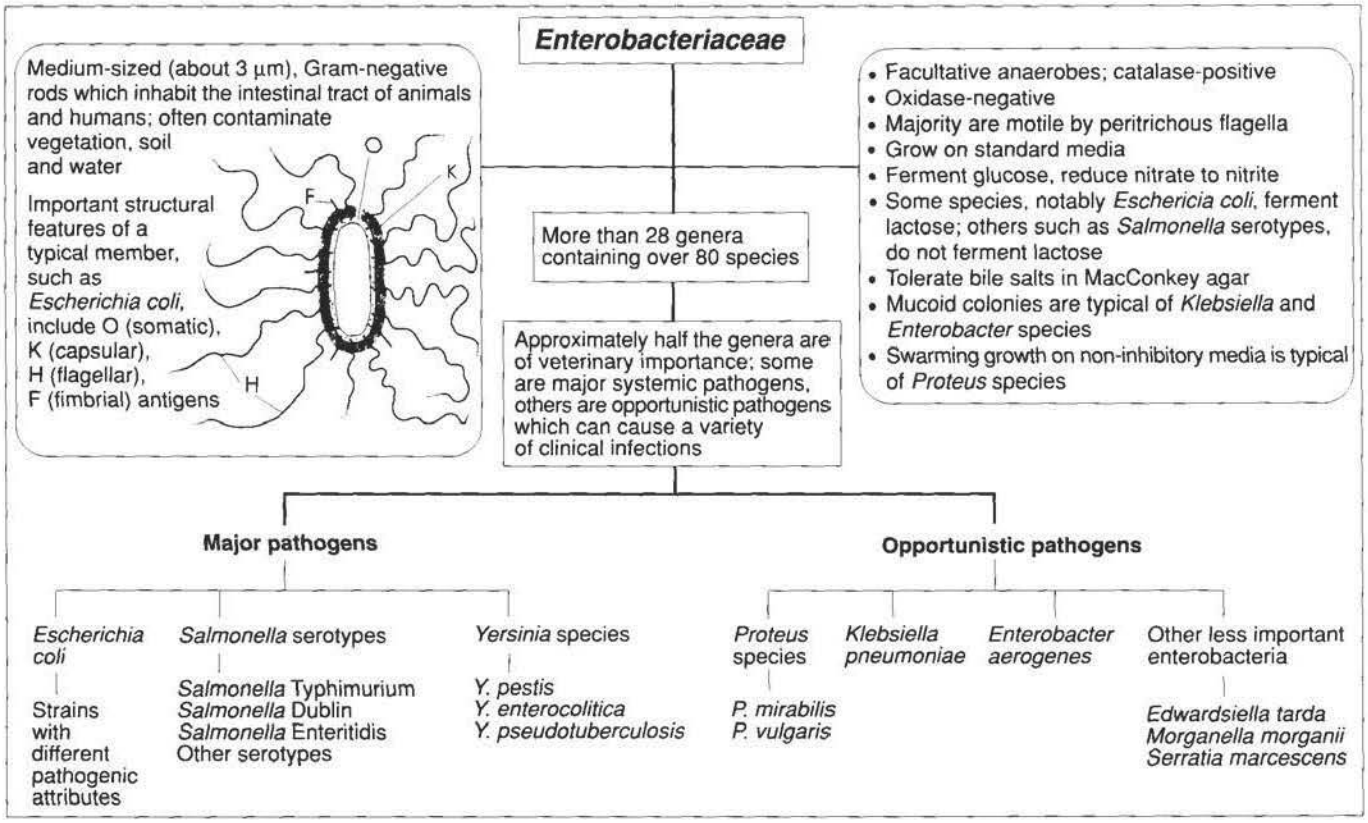


Paratuberculosis (Johne's disease)

This chronic, contagious, invariably fatal enteritis, which can affect domestic and wild ruminants, is caused by infection with *M. avium* subspecies *paratuberculosis*. Infection is acquired by calves at an early stage through ingestion of organisms shed in the faeces of infected animals. Under favourable conditions, *M. avium* subsp. *paratuberculosis* may remain viable in the environment for up to one year. Clinical disease is rarely encountered in cattle under two years of age. Ingested mycobacteria, engulfed by macrophages in which they survive and replicate, are found initially in Peyer's patches. Cell-mediated reactions are mainly responsible for the enteric lesions. As the disease progresses, an immune-mediated granulomatous reaction develops, with marked lymphocyte and macrophage accumulation in the lamina propria and submucosa. The resulting enteropathy leads to loss of plasma proteins and malabsorption of nutrients and water.

Affected cattle are usually more than two years of age when signs are first observed. The main clinical feature is diarrhoea, initially intermittent but becoming persistent and profuse. Progressive weight loss occurs in cattle despite an unaltered appetite. The mucosa of affected areas of the terminal small intestine and the large intestine of cattle is usually thickened and folded into transverse corrugations. The mesenteric and ileocaecal lymph nodes are enlarged and oedematous. Specimens for direct microscopy from live animals include scrapings or pinch biopsies from the rectum. Faeces may be submitted for culture and serum for serological tests. Postmortem specimens for histopathological examination from cattle include tissue from affected regions of the intestines and from regional lymph nodes. Isolation of *M. avium* subsp. *paratuberculosis* from faeces or tissues is difficult and time-consuming. Cell-mediated responses using johnin, the counterpart of tuberculin PPD, may be used as a field test. DNA probes, which are highly sensitive, are being used to detect the mycobacteria in faeces. Animals with clinical signs suggestive of paratuberculosis should be isolated. If the condition is confirmed, affected animals should be slaughtered promptly.

18 *Enterobacteriaceae* 1



Bacteria belonging to the family *Enterobacteriaceae* are Gram-negative rods which ferment glucose and other sugars and are oxidase-negative. They are catalase-positive, non-spore-forming facultative anaerobes which grow well on MacConkey agar. These enteric bacteria reduce nitrates to nitrites and some, such as *Escherichia coli*, ferment lactose. The majority of members are motile. The family contains more than 28 genera and over 80 species. Less than half of the genera are of veterinary importance.

Enterobacteria inhabit the intestinal tract of animals and humans and contaminate vegetation, soil and water. They can be arbitrarily grouped in three categories: major pathogens, opportunistic pathogens and non-pathogens. Opportunistic pathogens such as *Proteus* species, *Klebsiella pneumoniae* and *Enterobacter aerogenes* occasionally cause clinical disease in locations other than the alimentary tract. The major animal pathogens *E. coli*, *Salmonella* species and *Yersinia* species can cause both enteric and systemic disease. The main criteria for differentiating pathogenic members are presented in Table 18.1. Apart from some strains of *E. coli*, few enterobacteria produce haemolysis on blood agar. Lactose fermentation in MacConkey agar is an important identifying feature of *E. coli*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*. Colonies of these organisms and the surrounding medium are pink, due to acid production from lactose. *Proteus* species produce characteristic swarming on non-inhibitory media such as blood agar. Mucoid colonies are typical of *Klebsiella* and *Enterobacter* species. Reactions in triple sugar iron (TSI) agar are used primarily to confirm that colonies isolated on brilliant green agar or on XLD media are those of salmonellae. Lysine decarboxylase production is used to distinguish *Proteus* species from *Salmonella* species. *Proteus* species are negative whereas *Salmonella* species are positive in this test. The IMViC (indole production, methyl red test, Voges-Proskauer test, citrate utilization) tests are used to differentiate *E. coli* from other lactose fermenters (Table 18.1). Serotyping, using slide agglutination tests with antisera, are used to detect the somatic (O) and flagellar (H) antigens of *E. coli*, *Salmonella* and *Yersinia* species. This procedure allows identification of the organisms involved in disease outbreaks. Molecular techniques, usually based on nucleic acid analyses, are used in reference laboratories for differentiating enterobacteria.

Opportunistic members of the *Enterobacteriaceae* are sometimes involved in localized opportunistic infections in diverse anatomical locations. Faecal contamination of the environment contributes to the occurrence of opportunistic infection. The clinical conditions arising from infection with these opportunistic members are presented in Table 18.2. *Klebsiella pneumoniae* and *Enterobacter aerogenes* are two opportunistic pathogens commonly encountered in coliform mastitis in dairy cattle. These organisms usually gain entry to the mammary gland from contaminated environmental sources.

Table 18.1 The clinical relevance, growth characteristics and biochemical reactions of important members of the *Enterobacteriaceae*.

	Escherichia coli	*Salmonella* serotypes	*Yersinia* species	*Proteus* species	*Enterobacter aerogenes*	*Klebsiella pneumoniae*
Clinical importance	Major pathogen	Major pathogens	Major pathogens	Opportunistic pathogens	Opportunistic pathogen	Opportunistic pathogen
Cultural characteristics	Some strains haemolytic	–	–	Swarming growth[a]	Mucoid	Mucoid
Motility at 30°C	Motile	Motile	Motile[b]	Motile	Motile	Non-motile
Lactose fermentation	+	–	–	–	+	+
IMViC tests						
Indole production	+	–	v	±[c]	–	–
Methyl red test	+	+	+	+	–	–
Voges-Proskauer test	–	–	–	v	+	+
Citrate utilization test	–	+	–	v	+	+
H_2S production in TSI agar	–	+	–	+	–	–
Lysine decarboxylase	+	+	–	–	+	+

a when cultured on non-inhibitory medium
b except *Y. pestis*
c *P. vulgaris* +; *P. mirabilis* –
v reaction varies with individual species

Klebsiella pneumoniae is also reported to be one of the commonest causes of metritis in mares. *Proteus* species and *Klebsiella* species cause infections of the lower urinary tract in dogs. *Proteus* species are often implicated in otitis externa in dogs and sometimes in cats.

Yersinia species

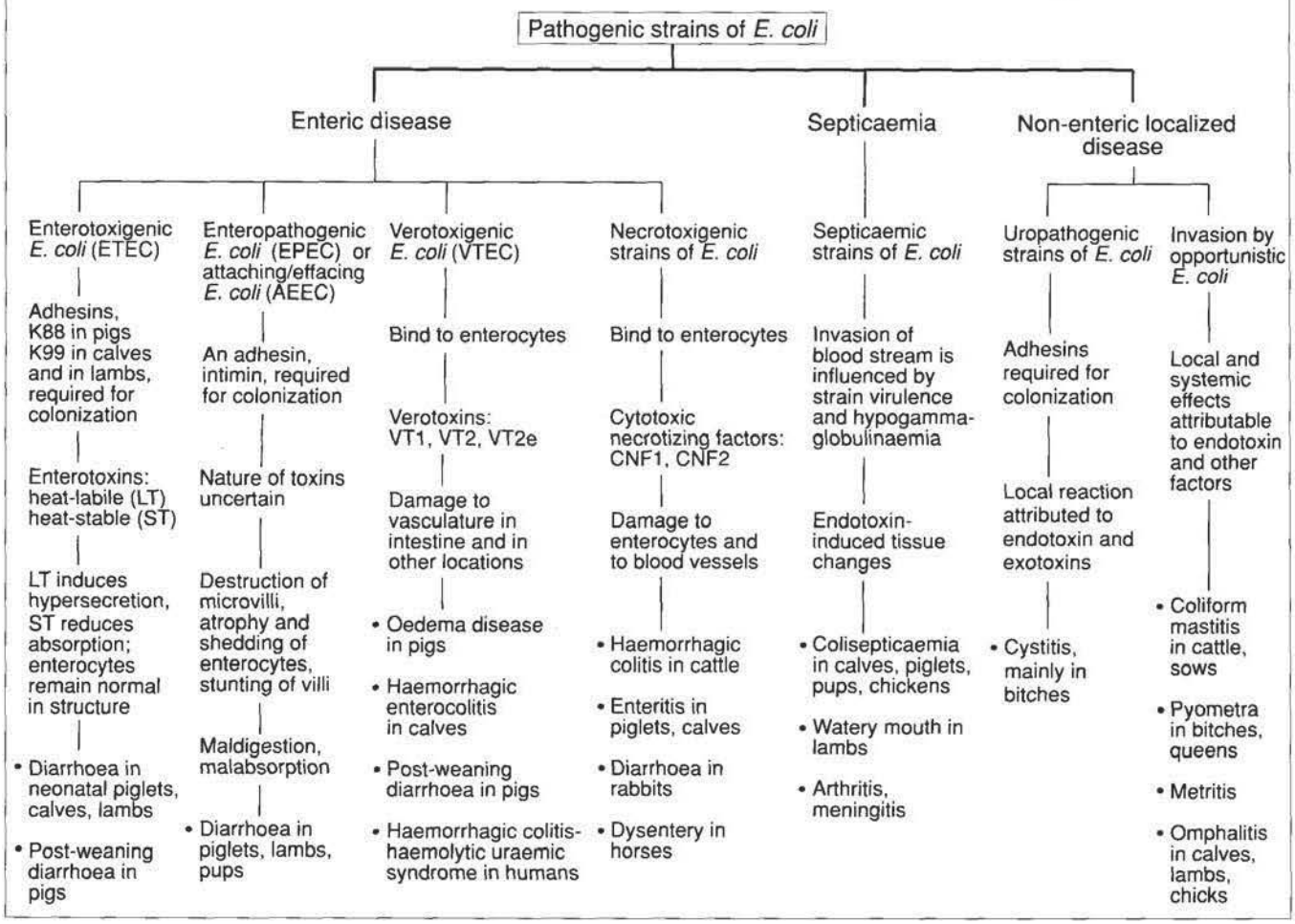
Yersinia species are non-lactose fermenters and, with the exception of *Y. pestis*, are motile. Although there are more than 10 *Yersinia* species, only *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis* are pathogenic for animals and humans. *Yersinia pestis*, which causes bubonic and pneumonic plague ('black death'), is more invasive than *Y. pseudotuberculosis* and *Y. enterocolitica*, and possesses additional virulence factors including an anti-phagocytic protein capsule and a plasminogen activator which aids systemic spread. In endemic areas, wild rodents are important reservoirs of *Y. pestis*. Fleas, especially *Xenopsylla cheopis*, the oriental rat flea, transmit infection to humans and other animals. Feline plague, caused by *Y. pestis*, usually occurs when cats ingest infected rodents. Three clinical forms of the disease are recognized: bubonic, pneumonic and septicaemic. The most common form of the disease is characterized by enlarged lymph nodes (buboes) associated with lymphatic drainage from the site of infection. Septicaemia may occur without lymphadenopathy and is potentially fatal. In endemic areas, cats and dogs should be routinely treated for fleas and rodent control measures should be implemented. *Yersinia pseudotuberculosis* causes enteric infections in a wide variety of wild and domestic animals. A septicaemic form of the disease occurs in laboratory rodents and in caged birds.

Table 18.2 Opportunistic pathogens in the *Enterobacteriaceae* and their associated clinical conditions.

Bacterial species	Clinical conditions
Enterobacter aerogenes	Coliform mastitis in cows and sows
Klebsiella pneumoniae	Coliform mastitis in cows; endometritis in mares; pneumonia in calves and foals; urinary tract infections in dogs
Proteus mirabilis and *P. vulgaris*	Urinary tract infections in dogs and horses; associated with otitis externa in dogs

19 *Enterobacteriaceae 2*

Pathogenic strains of *Escherichia coli*, their virulence factors and diseases which they produce



Escherichia coli

Colonization of the mammalian intestinal tract by *E. coli* from environmental sources occurs shortly after birth, and these organisms persist as important members of the normal flora of the intestine throughout life. Most strains of *E. coli* are of low virulence; pathogenic strains possess virulence factors which allow them to colonize mucosal surfaces and subsequently produce disease. Predisposing factors which permit colonization include age, immune status, nature of diet and heavy exposure to pathogenic strains. The main categories of pathogenic strains of *E. coli* and their clinical effects are illustrated. In recent years, *E. coli* O157:H7 has emerged as a major foodborne zoonotic pathogen in humans, responsible for the haemorrhagic colitis-haemolytic uraemic syndrome.

Clinical infections in young animals may be limited to the intestines (enteric colibacillosis, neonatal diarrhoea), or may manifest as septicaemia or toxaemia. In older pigs, post-weaning enteritis and oedema disease are manifestations of toxaemia. Non-enteric localized infections in adult animals, many due to opportunistic invasion, can involve the urinary tract, mammary glands and uterus. The virulence factors of pathogenic strains of *E. coli* include capsules, endotoxin, structures responsible for colonization, enterotoxins and other secreted products. Capsular polysaccharides, which are produced by some strains of *E. coli*, interfere with the phagocytic uptake of these organisms. Endotoxin, a lipopolysaccharide (LPS) component of the cell wall of Gram-negative organisms is released on death of the bacteria. The role of LPS in disease production includes pyrogenic activity, endothelial damage leading to disseminated intravascular coagulation, and endotoxic shock. Fimbrial adhesins, which are present on many enterotoxigenic strains of *E. coli*, allow attachment to mucosal surfaces in the small intestine. The most significant adhesins in strains of *E. coli* producing disease in domestic animals are K88 (F4), K99 (F5), 987P (F6) and F41. The most common adhesin present in strains of *E. coli* infecting

pigs is K88. The K99 and F41 adhesins occur in calves and K99 in lambs. The pathological effects of infection with pathogenic *E. coli*, other than those attributed to endotoxin, derive mainly from the production of enterotoxins, verotoxins or cytotoxic necrotizing factors. Unlike enterotoxins, which affect only the functional activity of enterocytes, verotoxins and cytotoxic necrotizing factors can produce demonstrable cell damage at their sites of action. To prevent enteric colibacillosis, neonatal diarrhoea and colisepticaemia, newborn animals should receive ample amounts of colostrum shortly after birth. Colostral antibodies can prevent colonization of the intestine by pathogenic *E. coli*. A clean, warm environment should be provided for newborn animals. Vaccination is of value for a limited number of diseases caused by *E. coli*.

Salmonella serotypes

The genus *Salmonella* contains more than 2,400 serotypes. Serotyping is based on the identification of somatic (O) and flagellar (H) antigens using specific antisera. Salmonellae are usually motile and do not ferment lactose. These organisms occur worldwide and infect many mammals, birds and reptiles and are mainly excreted in faeces. Ingestion is the main route of infection. Organisms may be present in water, soil, animal feeds, raw meat and offal, and in vegetable material. The source of environmental contamination is invariably faeces. In poultry, some species such as *Salmonella* Enteritidis infect the ovaries, and the organisms can be isolated from eggs.

Salmonellosis is of common occurrence in domestic animals and the consequences of infection range from sub-clinical carrier status to acute fatal septicaemia. Some *Salmonella* serotypes such as *Salmonella* Pullorum in poultry, *Salmonella* Choleraesuis in pigs and *Salmonella* Dublin in cattle are relatively host-specific. In contrast, *Salmonella* Typhimurium has a comparatively wide host range. The *Salmonella* serotypes of importance in domestic animals and the consequences of infection are indicated in Table 19.1. *Salmonella* Dublin causes a variety of clinical effects in cattle of different ages including acute or chronic enteric disease, septicaemia and abortion. In calves, joint ill, osteomyelitis and terminal dry gangrene may follow septicaemia or enteric disease. The virulence of salmonellae relates to their ability to invade host cells, replicate in them and resist both digestion by phagocytes and destruction by the complement components of plasma. Salmonellae often localize in the mucosae of the ileum, caecum and colon, and in the mesenteric lymph nodes of infected animals. Latent infections, in which salmonellae are present in the gall bladder but are not excreted, also occur. Clinical disease may develop from subclinical and latent infections if affected animals are stressed.

Enterocolitis caused by salmonella organisms can affect most species of farm animals, irrespective of age. Acute disease is characterized by fever, depression, anorexia and profuse foul-smelling diarrhoea often containing blood, mucus and epithelial casts. Dehydration and weight loss follow and pregnant animals may abort. Severely affected young animals become recumbent and may die within a few days of acquiring infection.

Table 19.1 Salmonella serotypes of clinical importance and the consequences of infection.

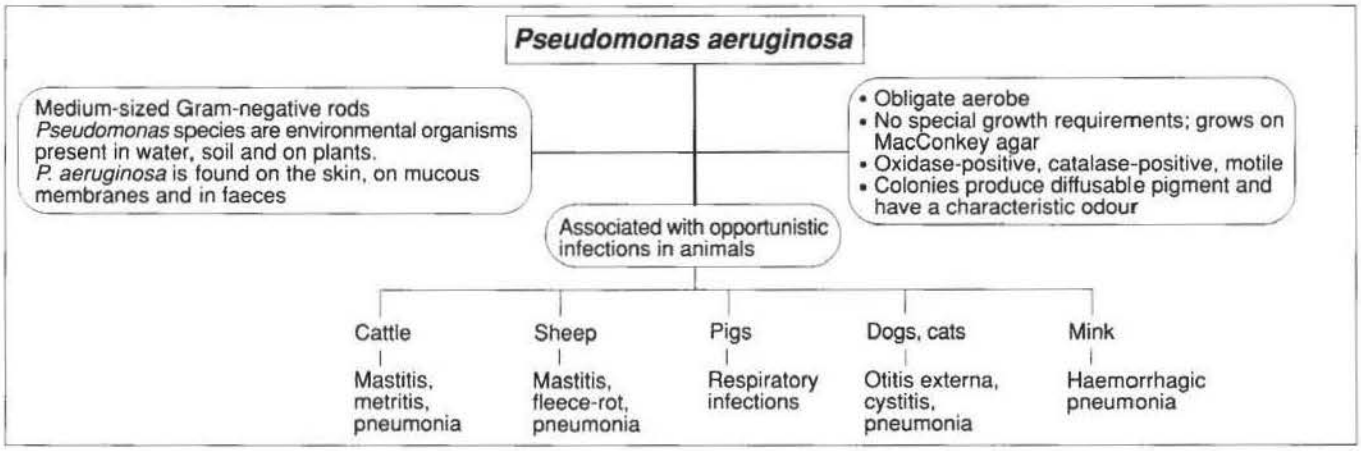
Salmonella serotype	Hosts	Consequences of infection
Salmonella Typhimurium	Many animal species	Enterocolitis and septicaemia
	Humans	Food poisoning
Salmonella Dublin	Cattle	Many disease conditions
	Sheep, horses, dogs	Enterocolitis and septicaemia
Salmonella Choleraesuis	Pigs	Enterocolitis and septicaemia
Salmonella Pullorum	Chicks	Pullorum disease (bacillary white diarrhoea)
Salmonella Gallinarum	Adult birds	Fowl typhoid
Salmonella Arizonae	Turkeys	Arizona or paracolon infection
Salmonella Enteritidis	Poultry	Often subclinical in poultry
	Many other species	Clinical disease in mammals
	Humans	Food poisoning
Salmonella Brandenburg	Sheep	Abortion

Septicaemic salmonellosis can occur in all age groups but is most common in calves, in neonatal foals and in young pigs. Onset of clinical disease is sudden with high fever, depression and recumbency. If treatment is delayed, many young animals with septicaemia die within 48 hours. In pigs with septicaemic *Salmonella* Choleraesuis infection, there is a characteristic bluish discolouration of the ears and snout.

Laboratory confirmation is required for salmonellosis. Specimens for submission should include faeces and blood from live animals. Intestinal contents and samples from tissue lesions should be submitted from dead animals and abomasal contents from aborted foetuses. Specimens should be cultured directly onto brilliant green and XLD agars and also added to selenite F or tetrathionate broth for enrichment and subculture. Suspicious colonies can be subjected to further biochemical tests and confirmed as salmonellae using commercially available specific antisera.

Control of salmonellosis is based on reducing the risk of exposure to infection by implementing a closed-herd policy, purchasing animals from reliable sources and preventing contamination of foodstuffs and water. Vaccination procedures for enhancing resistance and reducing the likelihood of clinical disease are used in cattle, sheep, pigs and poultry.

20 *Pseudomonas aeruginosa* and *Burkholderia* species



Pseudomonas aeruginosa

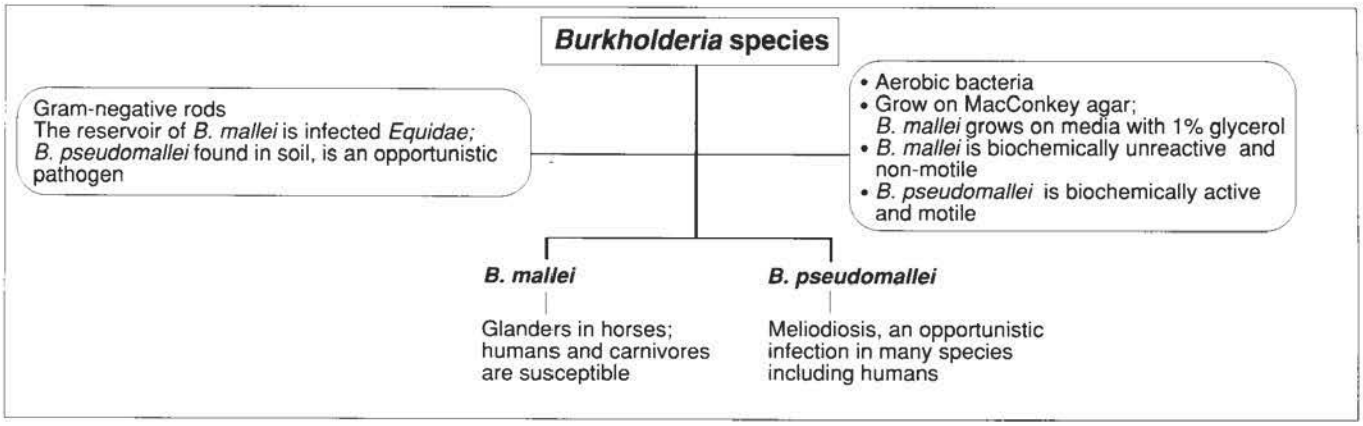
Pseudomonas species are environmental organisms which occur worldwide in water and soil, and on plants. *Pseudomonas aeruginosa* is a Gram-negative, obligate aerobe. Most isolates are oxidase-positive and catalase-positive. *Pseudomonas aeruginosa* is motile and can produce up to four diffusable pigment. Pyocyanin (blue-green), unique to this organism, is produced by most strains and specifically identifies *P. aeruginosa*. In common with other *Pseudomonas* species, *P. aeruginosa* is an environmental organism but it is also found on the skin, on mucous membranes and in faeces.

Pathogenic strains of *P. aeruginosa* produce a variety of toxins and enzymes which promote tissue invasion and damage. Attachment to host cells is mediated by fimbriae. Colonization and replication are aided by exoenzymes, extracellular slime and outer-membrane lipopolysaccharides. Tissue damage is caused by toxins such as exotoxin A, phospholipase C and proteases. The cytoplasmic membranes of neutrophils are damaged by a leukocidin. Host defence mechanisms against *P. aeruginosa* include opsonizing antibodies and phagocytosis by macrophages.

Pseudomonas aeruginosa causes a wide range of opportunistic infections (Table 20.1). Although predisposing factors are associated with the occurrence of many of these infections, some species such as farmed mink, appear to be particularly susceptible to the organism. Haemorrhagic pneumonia and septicaemia, caused by *P. aeruginosa*, occur sporadically in ranched mink with mortality rates up to 50% in some outbreaks. Bovine mastitis associated with this organism is often linked to udder washing with contaminated water, the insertion of contaminated intramammary antibiotic tubes or contaminated wipes. Fleece-rot of sheep, a condition associated with heavy or prolonged rainfall, has been reported from the UK and Australia. Maceration of the skin following water penetration of the fleece allows colonization by *P. aeruginosa*, resulting in suppurative dermatitis. Specimens suitable for laboratory examination include pus, respiratory aspirates, mid-stream urine, mastitic milk and ear swabs. Blood agar and MacConkey agar plates, inoculated with suspect material, are incubated aerobically at 37°C for 24 to 48 hours. On blood agar, the large flat colonies with a characteristic grape-like odour resemble those of some *Bacillus* species. Pyocyanin production is evident on both media. On MacConkey agar, lactose is not fermented.

Table 20.1 Clinical conditions arising from infection with *Pseudomonas aeruginosa*.

Host	Disease condition
Cattle	Mastitis, metritis, pneumonia, dermatitis, enteritis (calves)
Sheep	Mastitis, fleece-rot, pneumonia, otitis media
Pigs	Respiratory infections, otitis
Horses	Genital tract infections, pneumonia, ulcerative keratitis
Dogs, cats	Otitis externa, cystitis, pneumonia, ulcerative keratitis
Mink	Haemorrhagic pneumonia, septicaemia
Chinchillas	Pneumonia, septicaemia
Reptiles (captive)	Necrotic stomatitis



Burkholderia mallei and *Burkholderia pseudomallei*

Burkholderia species, previously classified in the genus *Pseudomonas*, include *B. mallei*, the cause of glanders and *B. pseudomallei*, the cause of melioidosis. Both diseases are zoonoses. *Burkholderia pseudomallei*, which is found in soil, occasionally infects animals and humans. Wild rodents can act as reservoirs for this organism. Although *B. mallei* can survive in the environment for up to six weeks, its reservoir is infected *Equidae*. These pathogens are Gram-negative rods which are obligate aerobes. Most isolates are oxidase-positive and catalase-positive. *Burkholderia pseudomallei* is motile but *B. mallei* is non-motile.

Glanders

This contagious disease of *Equidae*, caused by *B. mallei*, is characterized by the formation of nodules and ulcers in the respiratory tract or on the skin. Humans and carnivores are susceptible to infection. Glanders has been eradicated from most developed countries but sporadic cases of the disease occur in the Middle East, India, Pakistan, China and Mongolia.

Transmission follows ingestion of food or water contaminated with the nasal discharges of infected *Equidae*. Less commonly, infection may be acquired by inhalation or through skin abrasions. An acute septicaemic form of the disease is characterized by fever, nasal discharge and respiratory signs. Death usually follows within weeks. Chronic disease is more common and presents as nasal, pulmonary and cutaneous forms, all of which may be observed in an affected animal. In the nasal form, ulcerative nodules develop on the mucosa of the nasal septum and turbinates. A purulent, blood-stained nasal discharge is usually present. The respiratory form is characterized by respiratory distress and tubercle-like lesions in the lungs. The cutaneous form, termed farcy, is a lymphangitis in which nodules occur along the lymphatic vessels of the limbs. Ulcers develop and discharge a yellowish pus. Chronically affected horses may die after several months or may recover and continue to shed the organisms from the respiratory tract or skin. The presence of *B. mallei* in the host gives rise to a hypersensitivity reaction, the basis of the mallein test.

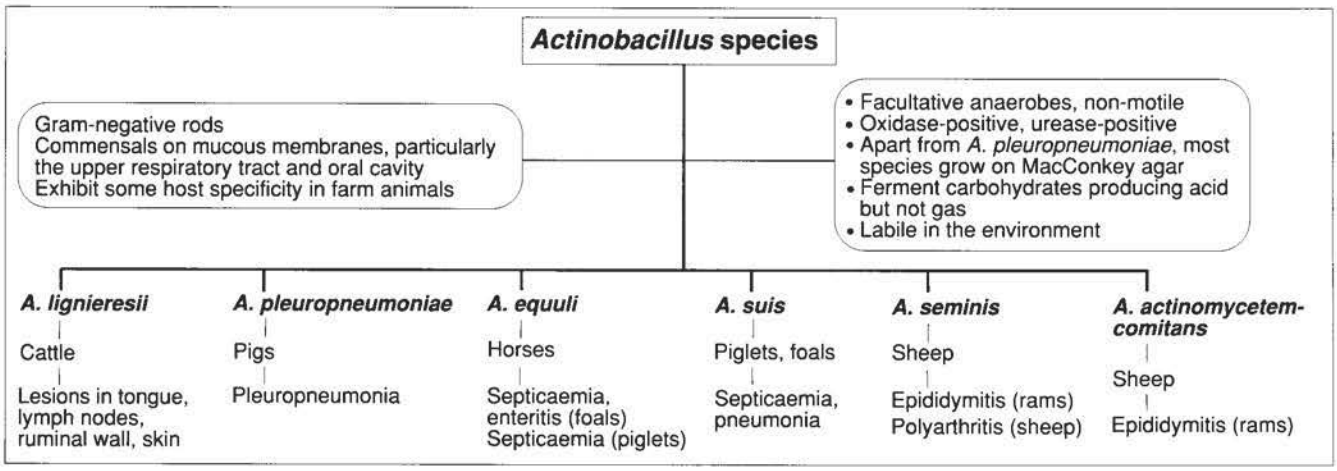
In regions where the disease is endemic, clinical signs may be diagnostic. Specimens for laboratory diagnosis, such as discharges from lesions, must be processed in a biohazard cabinet. *Burkholderia mallei* grows on MacConkey agar without utilizing lactose; it is comparatively unreactive and non-motile. The mallein test is an efficient field test for confirmation of glanders in horses. A test and slaughter policy is enforced in countries where the disease is exotic.

Melioidosis

This disease, caused by *B. pseudomallei*, is endemic in tropical and subtropical regions of Asia and Australia where the organism is widely distributed in soil and water. Infection may follow ingestion, inhalation or skin contamination from environmental sources. Many animal species including humans are susceptible. Melioidosis is a chronic, debilitating disease with a long incubation period. Abscesses may develop in many organs including the lungs, liver, spleen, joints and central nervous system. In horses, melioidosis can mimic glanders.

Specimens for laboratory diagnosis should include pus from abscesses. A biohazard cabinet must be used for processing specimens. Identification criteria for isolates include colonial appearance on blood agar and MacConkey agar (lactose is utilized in MacConkey agar), biochemical characteristics and agglutination by specific antiserum. In countries where the disease is exotic, confirmation of infection is followed by slaughter of infected animals.

21 *Actinobacillus* species



Actinobacillus species are non-motile, Gram-negative rods which occasionally have a coccobacillary appearence. These facultative anaerobes ferment carbohydrates, producing acid but not gas. Most species are urease-positive and oxidase-positive. Actinobacilli exhibit some host specificity and are mainly pathogens of farm animals. They are commensals on mucous membranes of animals, particularly in the upper respiratory tract and oral cavity. As actinobacilli cannot survive for long in the environment, carrier animals play a major role in transmission.

On primary isolation on blood agar, colonies of *A. lignieresii*, *A. equuli* and *A. suis* exhibit cohesive properties when touched with an inoculation loop. On MacConkey agar, *A. lignieresii*, *A. equuli* and *A. suis* grow well; *A. equuli* and *A. suis* ferment lactose, producing pink colonies (Table 21.1). Neither *A. pleuropneumoniae* nor *A. seminis* grow on MacConkey agar. Serotyping of *A. pleuropneumoniae* isolates is based on differences in capsular polysaccharide antigens.

Actinobacilli can cause a variety of infections in farm animals including 'timber (wooden) tongue' in cattle, pleuropneumonia in pigs, systemic disease in foals and piglets and epididymitis in rams.

Actinobacillosis in cattle

Actinobacillosis, a chronic pyogranulomatous inflammation of soft tissues, is most often manifest clinically in cattle as induration of the tongue, referred to as timber tongue. Lesions may also occur in the oesophageal groove and the retropharyngeal lymph nodes. The aetiological agent, *A. lignieresii*, is a commensal of the oral cavity and intestinal tract. The organisms enter tissues through erosions or lacerations in the mucosa and skin. The disease is usually sporadic. Animals with timber tongue have difficulty in eating and drool saliva. Involvement of the tissue of the oesophageal groove can lead to intermittent tympany. Localized pyogranulomatous lesions in the retropharyngeal lymph nodes are often found at slaughter.

Diagnosis is based on the history, induration of the tongue and a background of grazing rough pasture. Specimens for laboratory examination include pus, biopsy material and tissue from lesions at postmortem. Gram-negative rods are demonstrable in smears from exudates. Pyogranulomatous foci containing club colonies may be evident in tissue sections. Cultures on blood agar and MacConkey agar, incubated aerobically at 37°C for 72 hours, yield small, sticky, non-haemolytic colonies on blood agar and colonies which slowly ferment lactose on MacConkey agar. The identity of isolates can be confirmed by their biochemical profile and colonial appearance (Table 21.1). Treatment with sodium iodide parenterally, potassium iodide orally, potentiated sulphonamides or a combination of penicillin and streptomycin are usually effective.

Pleuropneumonia of pigs

Pleuropneumonia, caused by *A. pleuropneumonia* can affect susceptible pigs of all ages and occurs worldwide. This highly contagious disease affects pigs under 6 months of age. Virulent strains of the organism possess capsules which are both antiphagocytic and immunogenic. Fimbriae and other adhesins allow organisms to attach to cells of the respiratory tract. In addition, *A. pleuropneumoniae* produces three toxins which damage cell membranes.

Subclinical carrier pigs harbour the organism in their respiratory tracts and tonsils. Poor ventilation and sudden drops in ambient temperature seem to precipitate disease outbreaks. Aerosol transmission occurs in confined groups. In outbreaks of acute disease, some pigs may be found dead while others show dyspnoea, pyrexia and a disinclination to move. Blood-stained froth may be present around the nose and mouth and many pigs show cyanosis. Pregnant sows may abort. Morbidity rates may be up to 50%, with high mortality. Concurrent infections with *Pasteurella multocida* and mycoplasmas may

Table 21.1 Differentiating features of *Actinobacillus* species.

Feature	*A. lignieresii*	*A. pleuropneumoniae*	*A. equuli*	*A. suis*
Haemolysis on sheep blood agar	–	+	v	+
Colony type on blood agar	Cohesive	Not cohesive	Cohesive	Cohesive
Growth on MacConkey agar	+	–	+	+
CAMP test with *S. aureus*	–	+	–	–
Oxidase production	+	v	+	+
Catalase production	+	v	v	+
Urease production	+	+	+	+
Hydrolysis of aesculin	–	–	–	+
Acid from:				
L-arabinose	v	–	–	+
lactose	+	–	+	+
maltose	+	+	+	+
mannitol	+	v	+	–
melibiose	–	–	+	+
salicin	–	–	–	+
sucrose	+	+	+	+
trehalose	–	–	+	+

+ most isolates positive v variable reaction – most isolates negative

exacerbate the condition. Areas of consolidation and necrosis are found in the lungs at postmortem examination along with fibrinous pleurisy. Blood-stained froth may be present in the trachea and bronchi. A history of ventilation failure or environmental temperature decrease prior to an outbreak of disease is associated with pleuropneumonia. Specimens for laboratory examination should include tracheal washings or affected portions of lung tissue. Specimens, cultured on chocolate agar and blood agar, are incubated in an atmosphere of 5% to 10% CO_2 at 37°C for 72 hours. Identification criteria for isolates include small colonies surrounded by clear haemolysis, absence of growth on MacConkey agar and a positive CAMP test with *Staphylococcus aureus*. The biochemical profile of the isolates is also used for identification (Table 21.1). Twelve serotypes and two biotypes are recognized. Some biotypes require V factor, supplied by chocolate agar, for growth.

Chemotherapy should be based on the results of antibiotic susceptibility testing as antibiotic resistance is encountered in some strains. Polyvalent bacterins may induce protective immunity but do not prevent development of a carrier state. A subunit vaccine containing toxoids of three *A. pleuropneumoniae* toxins and capsular antigen has been developed. Predisposing factors such as poor ventilation, chilling and overcrowding should be avoided.

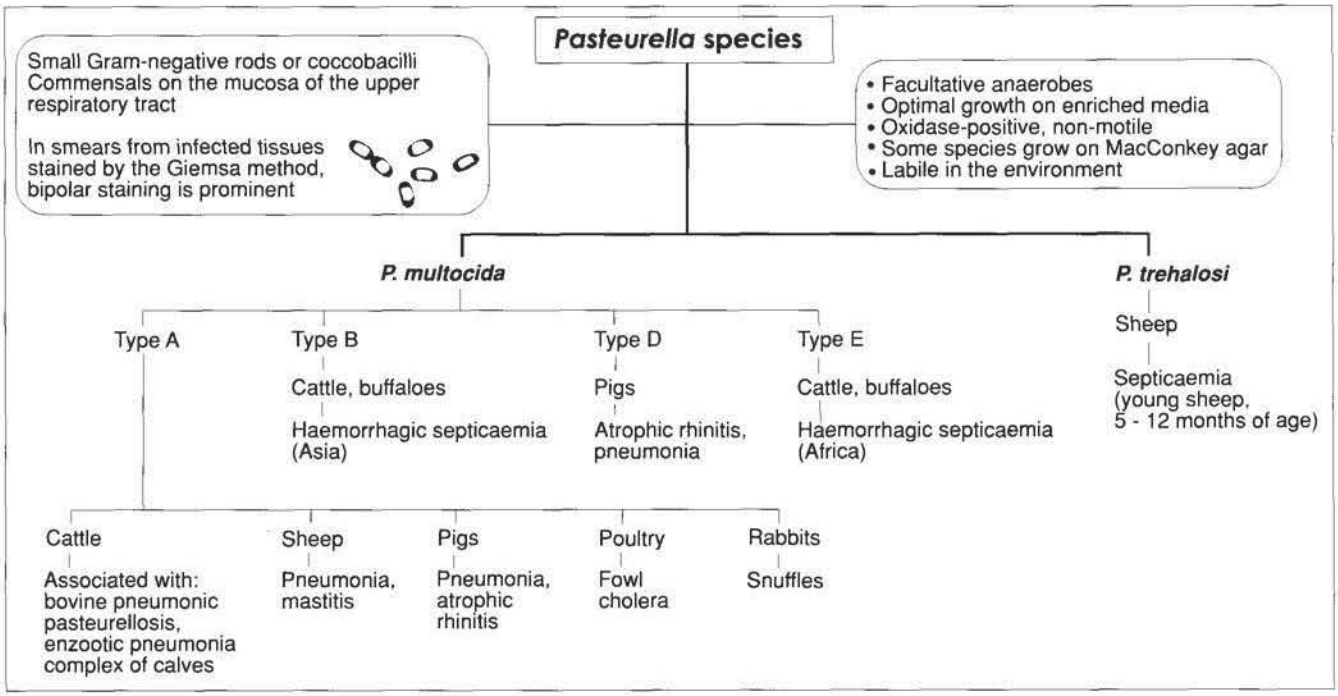
Sleepy foal disease

This is an acute, potentially fatal septicaemia of newborn foals caused by *A. equuli*. The organism is found in the reproductive and intestinal tract of mares. Foals can be infected *in utero* or after birth via the umbilicus. Affected foals are febrile and recumbent. Death usually occurs in one or two days. Foals which recover from the acute septicaemic phase may develop polyarthritis, nephritis, enteritis or pneumonia. Foals dying within 24 hours of birth have petechiation on serosal surfaces and enteritis. Those surviving for up to three days have typical pin-point suppurative foci in the kidneys. Specimens should be cultured on blood agar and MacConkey agar. Identification criteria for isolates include sticky colonies on blood agar, lactose-fermenting colonies on MacConkey agar and biochemical profiles (Table 21.1).

Other infections caused by actinobacilli

Actinobacillus suis can infect young pigs under three months of age. The disease is characterized by septicaemia and rapid death. Mortality may be up to 50% in some litters. Clinical signs include fever, respiratory distress and paddling of the forelimbs. *Actinobacillus seminis* is a common cause of epididymitis in young rams in New Zealand, Australia and South Africa.

22 *Pasteurella* species, *Mannheimia haemolytica* and *Francisella tularensis*

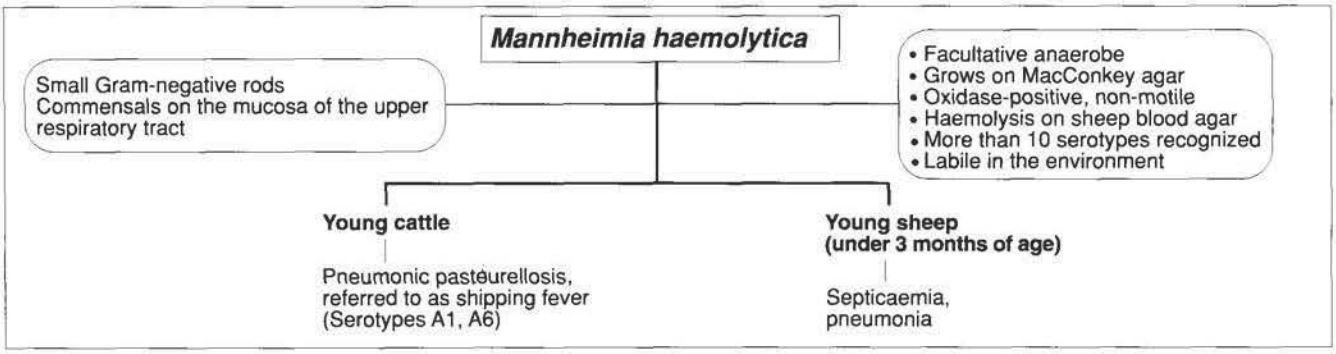


Pasteurella species and *Mannheimia haemolytica*

Pasteurella and *Mannheimia* species are small, non-motile, Gram-negative rods or coccobacilli. They are oxidase-positive facultative anerobes, and most species are catalase-positive. These organisms grow best on media supplemented with blood or serum. Some species, such as *Mannheimia haemolytica* and *Pasteurella trehalosi*, grow on MacConkey agar. In smears from infected tissues stained by the Giemsa method, pasteurellae exhibit bipolar staining. Most *Pasteurella* and *Mannheimia* species are commensals on the mucosae of the upper respiratory tract of animals.

Pasteurellae and *Mannheimia* species can be distinguished by colonial and growth characteristics and by biochemical reactions. Colonies of *P. multocida* are round, greyish, non-haemolytic and have a subtle characteristic odour. Colonies of *M. haemolytica* and *P. trehalosi* are haemolytic and odourless. Isolates of *P. multocida* are grouped into five serogroups, based on differences in their capsular polysaccharides. Seventeen serotypes of *M. haemolytica*/*P. trehalosi* are recognized on the basis of extractable surface antigens.

Many *P. multocida* infections are endogenous. The organisms may invade the tissues of immunosuppressed animals. Factors of importance in the development of disease include adhesion of the pasteurellae to the mucosa and avoidance of phagocytosis. Fimbriae may enhance mucosal attachment and the capsule, particularly in type A strains, has a major antiphagocytic role. Four main virulence factors have been identified in strains of *M. haemolytica* and *P. trehalosi*: fimbriae which may enhance colonization; a capsule that enhances survival in serum; endotoxin which can damage bovine leukocytes and endothelial cells; leukotoxin, a pore-forming



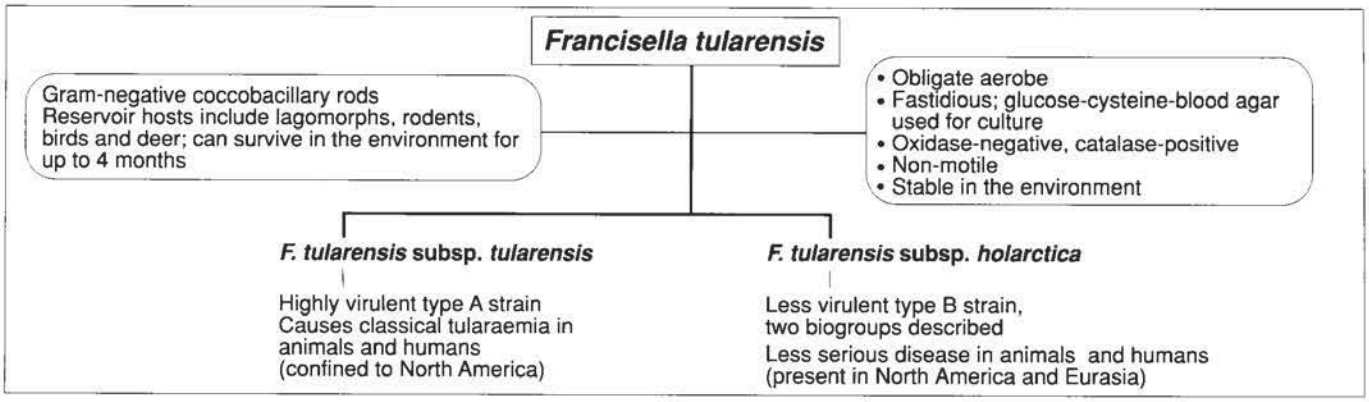
cytolysin that affects leukocyte and platelet function.

Clinical infections caused by pasteurellae and *Mannheimia* species in domestic animals are mainly attributed to *P. multocida*, *M. haemolytica* and *P. trehalosi* (Table 22.1). *Pasteurella multocida* has a wide host range, whereas *M. haemolytica* is largely restricted to ruminants, and *P. trehalosi* to sheep. The diseases associated with *P. multocida* infection include haemorrhagic septicaemia in ruminants, porcine atrophic rhinitis, fowl cholera and bovine pneumonic pasteurellosis. However, the main aetiological agent of bovine pneumonic pasteurellosis is *M. haemolytica*, and this organism is also responsible for pneumonia in sheep and septicaemia in young lambs. Bovine pneumonic pasteurellosis (shipping fever) occurs most commonly in young animals within weeks of being subjected to severe stress such as transportation, assembly in feedlots and close confinement. The condition is associated with *M. haemolytica*, serotype A1. Several respiratory viruses including parainfluenzavirus 3, bovine herpesvirus 1 and bovine respiratory syncytial virus may predispose to the bacterial invasion. Clinical signs include sudden onset of fever, depression, anorexia, tachypnoea and serous nasal discharge. In mixed infections, there is usually a marked cough and ocular discharge. At postmortem, the cranial lobes of the lungs are red, swollen and consolidated. Isolation of *M. haemolytica*, often in association with other pathogens, from bronchoalveolar lavage or affected lung tissue is confirmatory.

Outbreaks of ovine pneumonic pasteurellosis are usually caused by *M. haemolytica*, a commensal of the upper respiratory tract in a proportion of healthy sheep. Predisposing factors are poorly understood and flock outbreaks usually start with sudden deaths of some sheep and acute respiratory distress in others.

Table 22.1 The major pathogenic *Pasteurella* and *Mannheimia* species, their principal hosts and associated diseases.

***Pasteurella* species**	**Hosts**	**Disease conditions**
P. multocida		
type A	Cattle	Associated with bovine pneumonic pasteurellosis (shipping fever); associated with enzootic pneumonia complex of calves; mastitis (rare)
	Sheep	Pneumonia; mastitis
	Pigs	Pneumonia, atrophic rhinitis
	Poultry	Fowl cholera
	Rabbits	Snuffles
	Other animal species	Pneumonia following stress
type B	Cattle, buffaloes	Haemorrhagic septicaemia (Asia)
type D	Pigs	Atrophic rhinitis, pneumonia
type E	Cattle, buffaloes	Haemorrhagic septicaemia (Africa)
M. haemolytica (*P. haemolytica* biotype A)	Cattle	Bovine pneumonic pasteurellosis (shipping fever)
	Sheep	Septicaemia (under 3 months of age); pneumonia; gangrenous mastitis
P. trehalosi (*P. haemolytica* biotype T)	Sheep	Septicaemia (5 to 12 months of age)



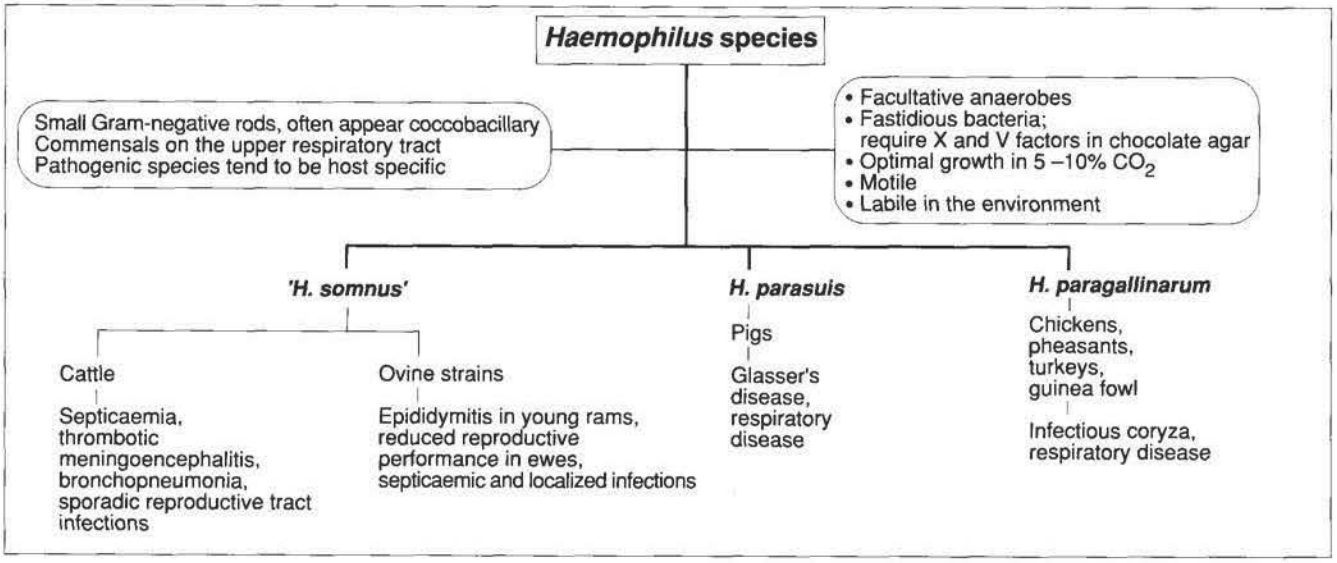
Francisella tularensis

Francisella tularensis is a poorly staining Gram-negative rod with a coccobacillary appearance. It is an obligate aerobe, non-motile and oxidase-negative. This fastidious organism requires the addition of cysteine to blood agar for growth. Highly virulent type A strains of *F. tularensis* subspecies *tularensis* occur only in North America. Less virulent type B strains of *F. tularensis* subspecies *holarctica* are found in Asia and North America. Reservoir hosts of *F. tularensis* include lagomorphs, rodents, birds and deer. Ticks and deerfly are important vectors in North America.

Outbreaks of tularaemia have been reported in sheep and other domestic animals. Transmission of infection often correlates with heavy tick infestation. The disease is characterized by fever, depression, inappetence, stiffness and other signs of septicaemia. Isolation procedures for *F. tularensis* must be carried out in a biohazard cabinet. A rising antibody titre in suspect animals is indicative of an active infection.

Tularaemia in humans, a serious and potentially fatal infection, often presents as a slow-healing ulcer accompanied by lymphadenopathy. Care is required when handling suspect animals or materials.

23 *Haemophilus* species and *Taylorella equigenitalis*



Haemophilus species

Haemophilus species are small Gram-negative rods which often appear coccobacillary. These motile organisms are facultative anaerobes which do not grow on MacConkey agar. They are fastidious bacteria which require one or both of the growth factors X (haemin) and V (nicotinamide adenine dinucleotide, NAD). Optimal growth occurs in an atmosphere of 5% to 10% CO_2 on chocolate agar which supplies both X and V factors. Small, transparent colonies are formed after incubation for 48 hours. *Haemophilus* species are commensals on the mucous membranes of the upper respiratory tract and do not survive for long periods away from their hosts.

The main pathogens in the genus are '*H. somnus*' in cattle and sheep, *H. parasuis* in pigs and *H. paragallinarum* in poultry (Table 23.1). More than 12 serotypes of *H. parasuis*, 15 serotypes of '*H. somnus*' and approximately 9 serotypes of *H. paragallinarum* have been identified. *Haemophilus* species are differentiated by requirements for X and V growth factors, by growth enhancement in an atmosphere of CO_2, by catalase and oxidase reactions and by carbohydrate utilization. Chocolate agar, which supplies both growth factors, is prepared by heating molten blood agar in a water bath at 80°C for about 10 minutes.

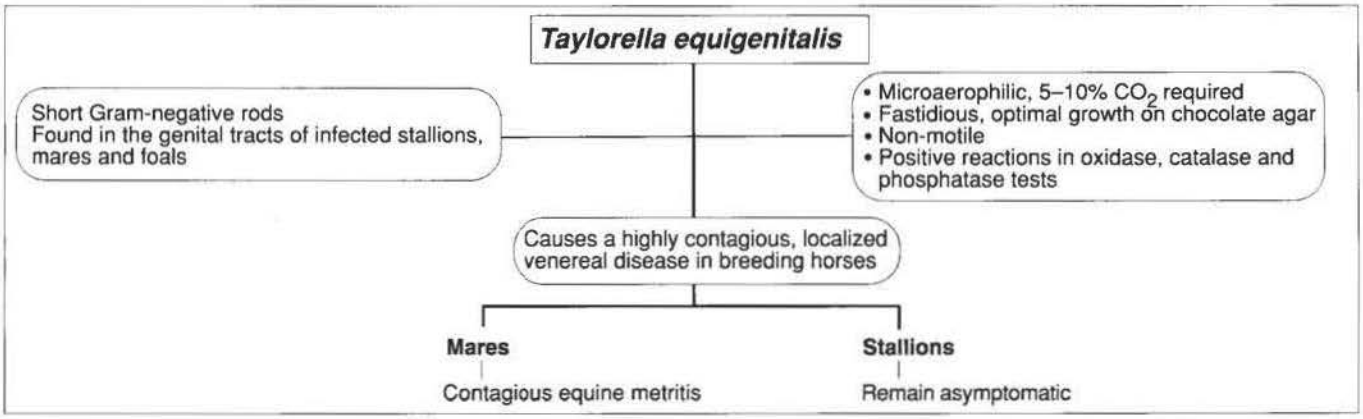
'*Haemophilus somnus*' is part of the normal bacterial flora of the male and female bovine genital tracts and it can also colonize the upper respiratory tract. Environmental stress factors contribute to the development of clinical disease. This organism is more resistant in the environment than most other *Haemophilus* species. Because septicaemia is commonly associated with '*H. somus*' infection, many organ systems may be involved. Thrombotic meningoencephalitis (TME), a common consequence of septicaemia, is encountered sporadically in young cattle recently introduced to feedlots. Some animals may be found dead and others may present with high fever and depression. Sudden death due to myocarditis has also been described. Severe neurological signs in young feedlot cattle may be indicative of TME. '*Haemophilus somnus*' is one of the pathogens commonly isolated from the enzootic calf pneumonia complex. Confirmation of the involvement of

Table 23.1 *Haemophilus* species of veterinary importance.

Haemophilus species	Hosts	Disease conditions
'*H. somnus*'	Cattle	Septicaemia, thrombotic meningoencephalitis, bronchopneumonia (in association with other pathogens), sporadic reproductive tract infections
'*H. somnus*' (ovine strains)	Sheep	Epididymitis in young rams; vulvitis, mastitis and reduced reproductive performance in ewes; septicaemia, arthritis, meningitis and pneumonia in lambs
H. parasuis	Pigs	Glasser's disease, secondary invader in respiratory disease
H. paragallinarum	Chickens Pheasants, turkeys, guinea fowl	Infectious coryza Respiratory disease

'*H. somnus*' in bovine infections requires the isolation and identification of the pathogen from cerebrospinal fluid or postmortem lesion material.

Glasser's disease, caused by *H. parasuis*, manifests as polyserositis and leptomeningitis, usually affecting pigs from weaning up to 12 weeks of age. Some cases present as polyarthritis. *Haemophilus parasuis* is part of the normal flora of the upper respiratory tract of pigs. The presence of maternally-derived antibodies prevents the development of clinical signs. How-ever, Glasser's disease may occur sporadically in two to four week old piglets subjected to stressful environmental conditions. Clinical signs develop two to seven days following exposure to stress factors such as weaning or transportation. Anorexia, pyrexia, lameness, recumbency and convulsions are features of the disease. Pigs may die suddenly without showing signs of illness. Postmortem findings may include fibrinous polyserositis, polyarthritis and meningitis. Isolation and identification of *H. parasuis* from joint fluid, heart blood, cerebrospinal fluid or postmortem tissues of a recently-dead pig is confirmatory. Commercially available bacterins or autogenous bacterins may stimulate serotype-specific protective immunity.



Taylorella equigenitalis

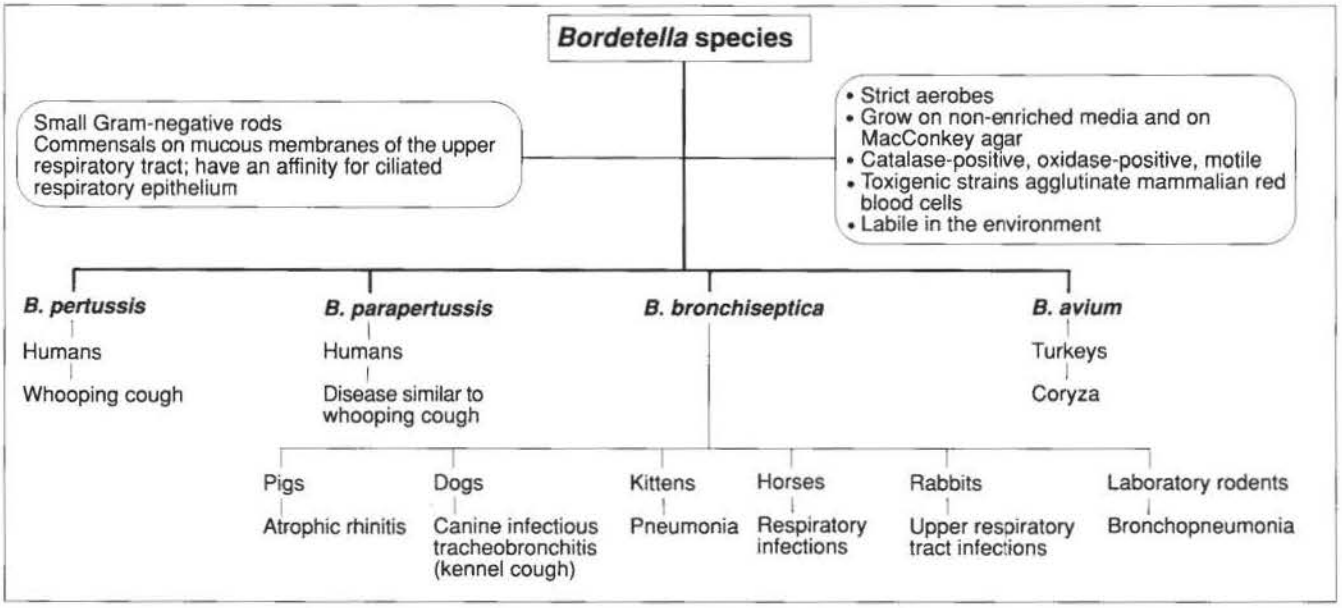
This organism, *Taylorella equigenitalis*, formerly known as *Haemophilus equigenitalis*, is a short, non-motile, Gram-negative rod which gives positive reactions in catalase, oxidase and phosphatase tests. It is microaerophilic, slow-growing and highly fastidious, requiring chocolate agar and 5% to 10% CO_2 for optimal growth. Although the bacterium is not dependent on the X or V factors, availability of factor X stimulates growth. *Taylorella equigenitalis*, the cause of contagious equine metritis (CEM), appears to infect only *Equidae*. The organism is found in the genital tract of stallions, mares and foals. Contagious equine metritis is a highly contagious, localized venereal disease characterized by mucopurulent vulval discharge and temporary infertility in mares. The condition is economically important because it disrupts breeding programmes on thoroughbred stud farms. Infected stallions and mares are the main reservoirs of infection. Transmission of the bacterium usually occurs during coitus although infection may be transferred by contaminated instruments. Foals born to infected dams may acquire infection *in utero* or during parturition.

Infected stallions and a minority of infected mares remain asymptomatic. Most affected mares develop a copious mucopurulent vulval discharge without systemic disturbance within a few days of service by a carrier stallion. The discharge may continue for up to two weeks and infected mares remain infertile for several weeks. Although some mares recover without treatment, up to 25% of mares remain carriers. Infection does not confer protective immunity and reinfection can occur. After introduction into the uterus, the pathogenic organisms replicate and induce an acute endometritis.

Diagnosis is based on the history of individual animals and laboratory tests. A copious, mucopurulent vulval discharge two to seven days after service may indicate the presence of CEM. Specimens for bacteriology should be collected before and during the breeding season. Swabs from mares should be taken from the clitoral fossa and sinuses, and from the endometrium at oestrus using a double-guarded swab. Swabs from stallions and teaser stallions are taken from the urethra, urethral fossa and penile sheath. Chocolate agar-based media are suitable for the isolation of *T. equigenitalis*. Inoculated plates are incubated in an atmosphere of 5% to 10% CO_2 for four to seven days. Identification criteria for isolates include small, yellowish-grey colonies giving positive catalase, oxidase and phosphatase tests. A slide agglutination test and the fluorescent antibody technique may be used to confirm the identity of the isolate. A polymerase chain reaction technique has been developed for detecting *T. equigenitalis* in specimens. If CEM is diagnosed on a stud farm, all breeding services should immediately cease.

Elimination of *T. equigenitalis* from both mares and stallions can be achieved by washing the external genitalia with a 20% solution of chlorhexidine, combined with local application of antimicrobial drugs. Contagious equine metritis is a notifiable disease in many countries with an advanced thoroughbred industry. No vaccine is available for the control of this disease.

24 *Bordetella bronchiseptica, Bordetella avium* and *Moraxella bovis*



Bordetella bronchiseptica and *B. avium*

The genus *Bordetella* contains four species, *B. pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*. *Bordetella pertussis*, the type species, and *B. parapertussis* are human pathogens associated with whooping cough in children. *Bordetella bronchiseptica* infects a wide range of animal species, while *B. avium* is a pathogen of avian species. The bordetellae are occasional pathogens with an affinity for ciliated respiratory epithelium. *Bordetella bronchiseptica* and *B. avium* are small, Gram-negative rods with a coccobacillary appearance.

Table 24.1 Differentiating features of *Bordetella bronchiseptica*, *B. avium* and *Alcaligenes faecalis*.

Feature	*B. bronchiseptica*	*B. avium*	*Alcaligenes faecalis*[a]
Colonial characteristics on:			
Sheep blood agar	Haemolysis	No haemolysis	No haemolysis
MacConkey agar	Pale, pinkish hue	Pale, pinkish hue	Pale
Selective medium	Small, blue	Small, blue	Large, greenish
Oxidase production	+	+	+
Catalase production	+	+	+
Urease production	+	–	–
Utilization of carbon exclusively from:			
Citrate	+	+	+
Malonate	–	–	+
Nitrate reduction	+	–	–
Motility	+	+	+
Haemagglutinating activity of virulent strains	Agglutination of ovine and bovine red blood cells	Agglutination of guinea-pig red blood cells	–

a an organism, not of veterinary significance, which may require differentiation from bordetellae

Table 24.2 *Bordetella* species of veterinary importance and disease conditions with which they are associated.

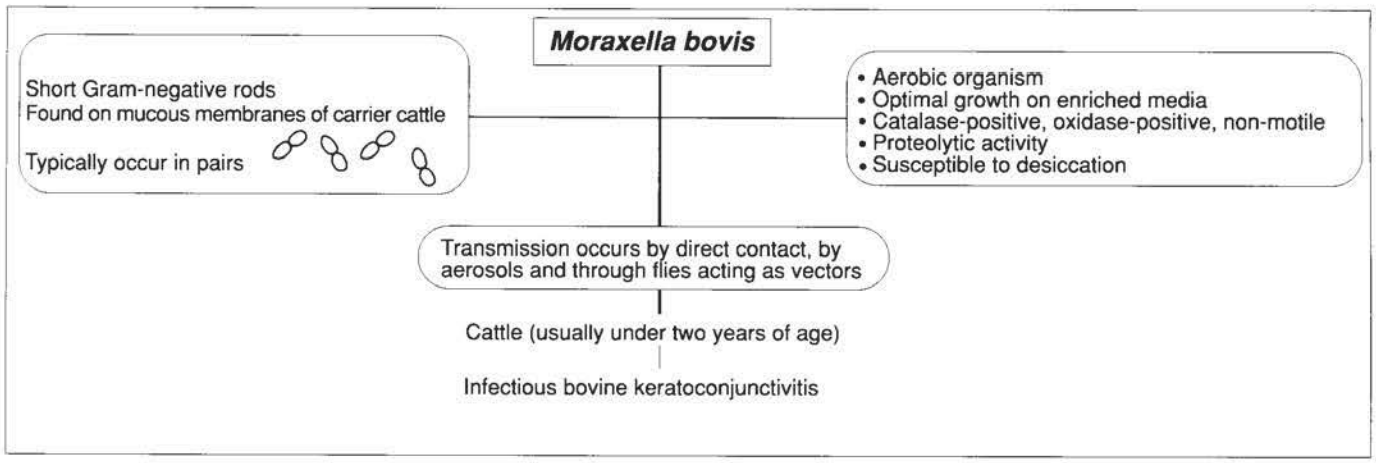
Bordetella species	Host	Disease conditions
B. bronchiseptica	Pigs	Atrophic rhinitis
	Dogs	Canine infectious tracheobronchitis
	Kittens	Pneumonia
	Horses	Respiratory infections
	Rabbits	Upper respiratory tract infection
	Laboratory rodents	Bronchopneumonia
B. avium	Turkeys	Coryza

They are catalse-positive, oxidase-positive aerobes and are motile peritrichous bacteria. They derive their energy mainly from oxidation of amino acids. They are commensals on the mucous membranes of the upper respiratory tract of animals and their survival time in the environment is short.

Bordetellae can be identified by growth characteristics, biochemical reactions and by their ability to agglutinate red blood cells (Table 24.1). *Bordetella avium* requires differentiation from *Alcaligenes faecalis*, which is non-pathogenic. Bordetellae exhibit phase changes which correlate with virulence and are identifiable by colonical appearance. Virulence is mediated by several factors including a filamentous haemagglutinin, tracheal cytotoxin, pertactin and fimbriae which allow attachment to cilia of the upper respiratory tract.

The diseases associated with *B. bronchiseptica* and *B. avium* are summarized in Table 24.2. Clinical signs associated with bordetellae usually relate to upper respiratory tract infection. Young animals are more susceptible than adults and stress predisposes to outbreaks of disease. Canine infectious tracheobronchitis (kennel cough) is one of the most prevalent respiratory complexes of dogs. The microbial pathogens implicated in kennel cough include *B. bronchiseptica*, canine parainfluenzavirus 2 and canine adenovirus 2. Transmission occurs through respiratory secretions, either by direct contact or by aerosols. Clinical signs, which include coughing, gagging or retching, develop within days of exposure. The disease, which may persist for up to 14 days, is usually self-limiting. Diagnosis is based on a history of recent exposure to carrier dogs and characteristic clinical signs. Modified live vaccines decrease the severity of clinical signs but may not prevent infection.

In pigs, infection with *B. bronchiseptica* may facilitate colonization by toxigenic *Pasteurella multocida* type D, with the subsequent development of severe atrophic rhinitis and distortion of the snout. Overstocking and poor ventilation can contribute to the development of atrophic rhinitis.

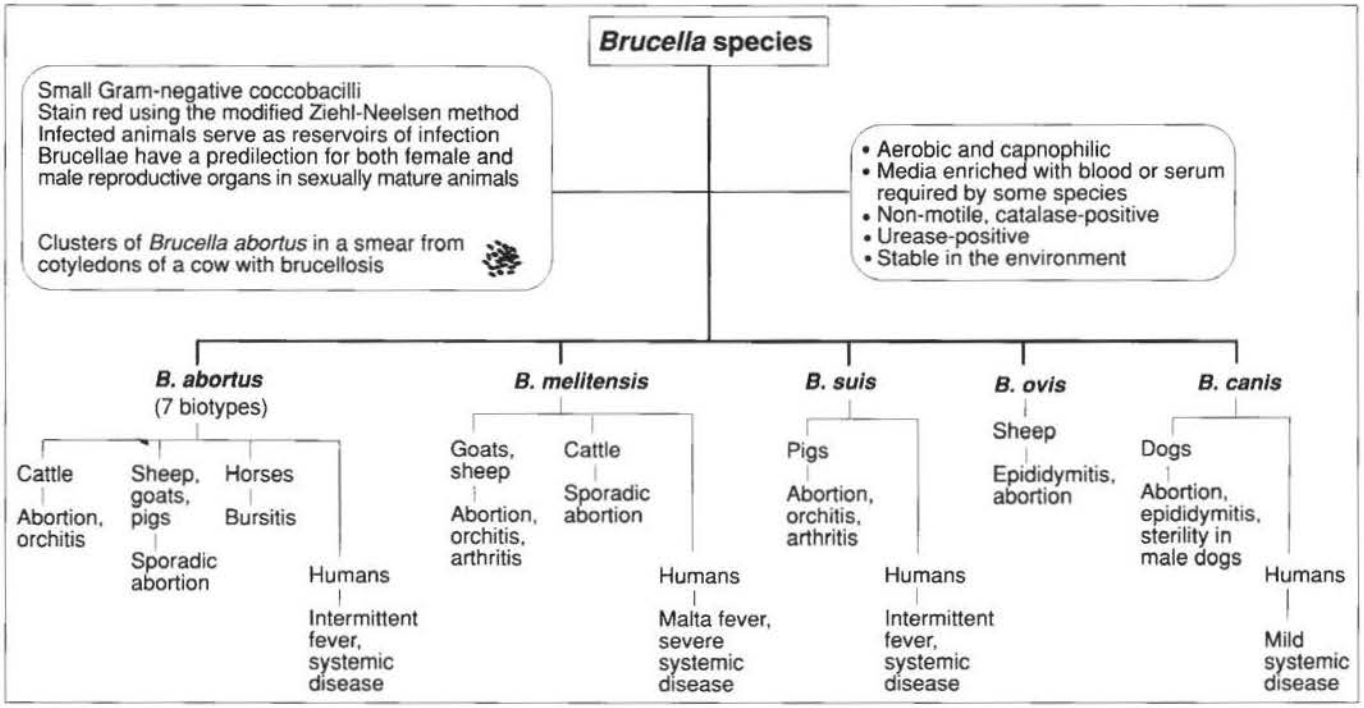


Moraxella bovis

Moraxella bovis occurs as short, plump Gram-negative rods or cocci, typically in pairs. This organism is non-motile, aerobic and usually catalase-positive and oxidase-positive. Growth of this proteolytic organism is enhanced by the addition of blood or serum to media. When isolated from cases of infectious bovine keratoconjunctivitis, virulent strains are fimbriate, haemolytic and grow into the agar. *Moraxella bovis* is found on mucous membranes of carrier animals. The organism, which is susceptible to desiccation, is short lived in the environment.

Infectious bovine keratoconjunctivitis, sometimes referred to as 'pink eye', is a highly contagious condition, caused by *M. bovis*, affecting the superficial structures of the eyes. Affected animals are usually under two years of age and there appears to be an age-related immunity. Transmission can occur by direct contact, by aerosols and through flies acting as vectors. The virulence of *M. bovis* is attributed to fimbriae which allow adherence of the organisms to the cornea in spite of lacrimal secretions and blinking. During bacterial replication, haemolysin and other lytic enzymes are produced. Strains which lack either haemolysin or fimbriae are avirulent. The disease initially manifests as blepharospasm, conjunctivitis and lacrimation. Progression of the condition through keratitis to corneal ulceration, opacity and abscessation may occasionally lead to panophthalmitis and permanent blindness. In most mild cases, the cornea heals within a few weeks although there may be permanent scarring of the structure. The disease, which characteristically affects a number of animals in a herd, can be diagnosed by its clinical presentation and confirmed by isolation and identification of the pathogen in lacrimal secretions.

25 *Brucella* species 1



Brucella species are small, non-motile, coccobacillary, Gram-negative bacteria. As they are not decolourized by 0.5% acetic acid in the modified Ziehl-Neelsen (MZN) technique, they are classed as MZN-positive. *Brucella* species are aerobic, capnophilic and catalase-positive. The growth of brucellae is enhanced in an atmosphere of 5% to 10% CO_2. Media enriched with blood or serum are required for culturing *B. abortus* and *B. ovis*. Most brucellae have a tropism for both female and male reproductive organs in sexually mature animals and each *Brucella* species tends to infect a particular animal species. Infected animals serve as reservoirs of infection which often persists indefinitely. Organisms, shed by infected animals, can remain viable in a moist environment for many months.

Brucella species are differentiated by colonial appearance, biochemical tests, specific cultural requirements and growth inhibition by dyes (Table 25.1). In addition, agglutination with monospecific sera and susceptibility to bacteriophages are employed for definitive identification.

Virulent brucellae, when engulfed by phagocytes on mucous membranes, are transported to regional lymph nodes. Brucellae persist within macrophages but not within neutrophils. Inhibition of phagosome-lysosome function is a major mechanism for intracellular survival and an important determinant of bacterial virulence. Intermittent bacteraemia results in spread and localization in the reproductive organs and associated glands in sexually mature animals. Erythritol, a polyhydric alcohol which acts as a growth factor for brucellae, is present in high concentrations in the placentae of cattle, sheep, goats and pigs. This growth factor is also found in other organs such as the mammary gland and epididymis, which are targets for brucellae. The hosts and clinical significance of *Brucella* species are presented in Table 25.2. Although each *Brucella* species has its own natural host, *B. abortus*, *B. melitensis* and biotypes of *B. suis* can infect animals other than their preferred hosts.

The diagnosis of brucellosis depends on serological testing and on isolation and identification of the infecting *Brucella* species. Care should be taken during collection and transportation of specimens, which should be processed in a biohazard cabinet. Specimens for laboratory examination should relate to the specific clinical condition encountered. MZN-stained smears from specimens, particularly cotyledons, foetal abomasal contents and uterine discharges often reveal MZN-positive coccobacilli. In specimens containing cells, the organisms appear in clusters. The polymerase chain reaction can be used to detect brucellae in tissues. A nutritious medium such as Columbia agar, supplemented with 5% serum and appropriate antimicrobial agents, is used for isolation. Plates are incubated at 37°C in 5 to 10% CO_2 for up to 5 days. Although CO_2 is a specific requirement for individual species, the majority of brucellae are capnophilic.

Serological testing is used for international trade and for identifying infected herds or flocks and individual animals in national eradication schemes. Brucellae share antigens with some other Gram-negative bacteria such as *Yersinia enterocolitica* serotype O:9, and consequently cross-reactions can occur in agglutination tests.

Table 25.1 Characteristics of *Brucella* species of veterinary importance.

Brucella species	Number of biotypes	Requirement for CO_2	Production of H_2S	Urease activity	Growth in media containing	
					Thionin (20 µg/ml)	Basic fuchsin (20 µg/ml)
B. abortus	7	v	v	+	v	v
B. melitensis	3	–	–	v	+	+
B. suis	5	–	v	+	+	v
B. ovis	1	+	–	–	+	–
B. canis	1	–	–	+	+	–

v variable reactions related to different biotypes

Porcine brucellosis

Porcine brucellosis, caused by *B. suis*, occurs occasionally in the USA but is more prevalent in Latin America and Asia. Infection is acquired by ingestion or by coitus and may be self-limiting in some animals. Clinical signs in sows include abortion, stillbirths, neonatal mortality and temporary sterility. Boars excreting brucellae in semen may either be clinically normal or present with testicular abnormalities. Associated sterility may be temporary or permanent. Lesions may also be found in bones and joints. The Rose-Bengal plate agglutination test and the indirect ELISA are the most reliable serological methods for the diagnosis of porcine brucellosis. A test and slaughter policy is the main control measure in countries where the disease is exotic.

Canine brucellosis

Infection with *Brucella canis* has been recorded in dogs in the USA, Japan, and Central and South America. Because of difficulties with diagnosis, the distribution of the disease may be more extensive than currently recognized. As *B. canis* is permanently in the rough form, it is of comparatively low virulence causing relatively mild and asymptomatic infections. In breeding establishments, infection may manifest clinically as abortions, decreased fertility, reduced litter sizes and neonatal mortality. Most bitches which have aborted subsequently have normal gestations. In male dogs the main clinical feature of the disease is infertility associated with orchitis and epididymitis. Infertility may be permanent and dogs with chronic infections are often aspermic. A rapid slide agglutination test kit containing 2-mercaptoethanol is used as a screening test. Confirmatory tests include a tube agglutination test, ELISA and an agar gel immunodiffusion test. Control is based on routine serological testing and removal of infected animals from breeding programmes.

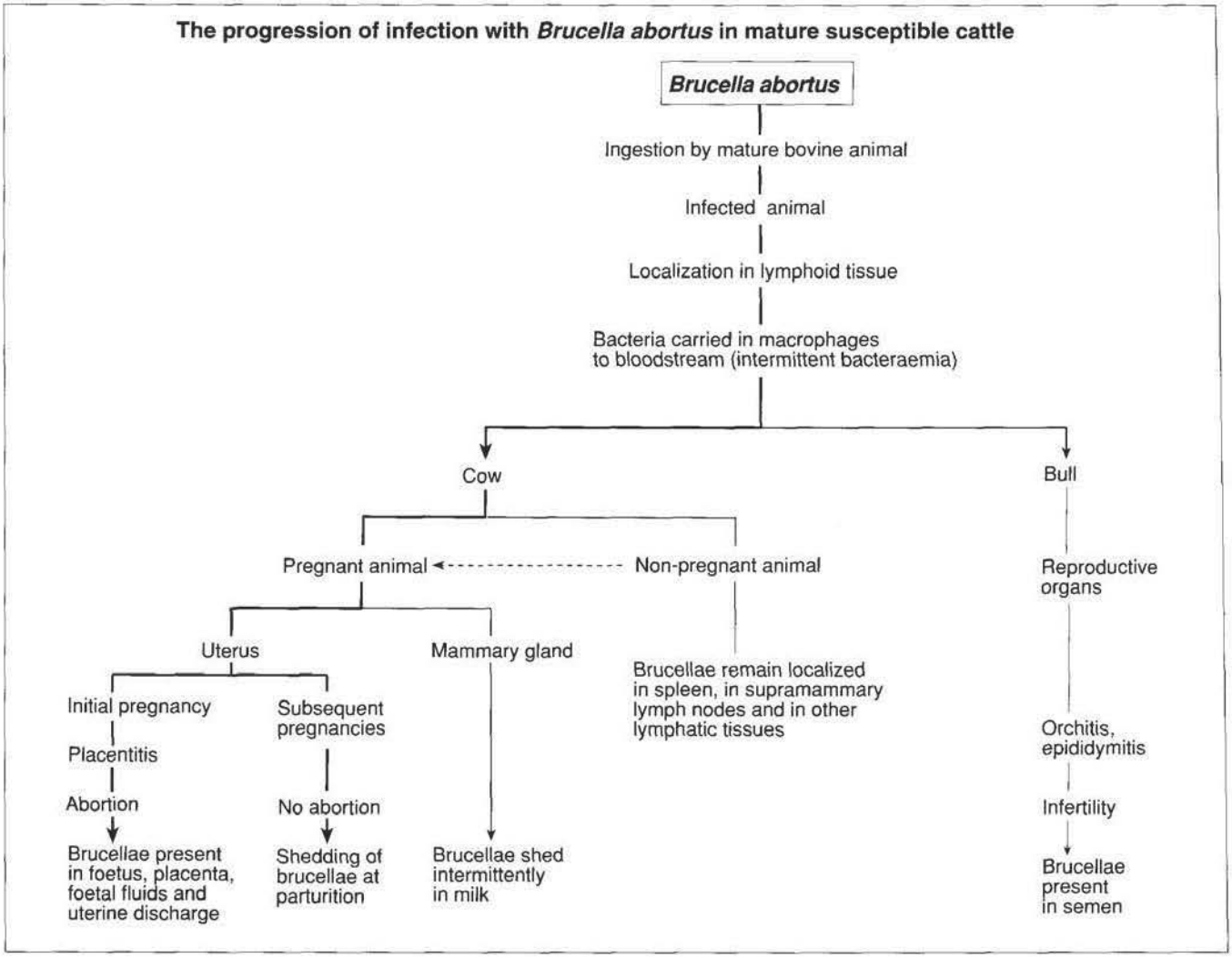
Ovine epididymitis caused by *B. ovis*

Brucella ovis causes infection in sheep which is characterized by epididymitis in rams and placentitis in ewes. The infection, which was first recorded in New Zealand and Australia, is now established in many other countries. The consequences of infection include reduced fertility in rams, sporadic abortion in ewes and increased perinatal mortality. *Brucella ovis* may be present in semen about five weeks after infection and epididymal lesions can be detected by palpation at about nine weeks. In countries where the disease is endemic, premating checks on rams include serological testing and scrotal palpation. The most efficient and widely-used serological tests for *B. ovis* are the agar gel immunodiffusion test, the complement fixation test and the indirect ELISA.

Table 25.2 *Brucella* species, their host range and the clinical significance of infection.

Brucella species	Usual host/clinical significance	Species occasionally infected/clinical significance
B. abortus	Cattle/abortion, orchitis	Sheep, goats, pigs/sporadic abortion Horses/bursitis Humans/intermittent fever, systemic disease
B. melitensis	Goats, sheep/abortion, orchitis, arthritis	Cattle/sporadic abortion, brucellae in milk Humans/Malta fever, severe systemic disease
B. suis	Pigs/abortion, orchitis, arthritis, spondylitis, infertility	Humans/intermittent fever, systemic disease
B. ovis	Sheep/epididymitis in rams, sporadic abortion in ewes	
B. canis	Dogs/abortion, epididymitis, discospondylitis, sterility in male dogs	Humans/mild systemic disease

26 *Brucella* species 2



Bovine brucellosis

Infection of cattle with *Brucella abortus* was formerly worldwide in distribution. National eradication programmes have reduced bovine brucellosis to a low level in many countries. Although acquired most often by ingestion, infection can occasionally follow venereal contact, penetration through skin abrasions, inhalation or transplacental transmission. Abortion storms may be encountered in herds with a high percentage of susceptible pregnant cows, Abortion usually occurs after the fifth month of gestation and subsequent pregnancies are usually carried to term. Large numbers of brucellae are excreted in foetal fluids for about two to four weeks following an abortion and at subsequent parturitions, although infected calves appear normal. Infection in calves is of limited duration, in contrast to cows, in which infection of the mammary glands and associated lymph nodes persists for many years. Brucellae may be excreted intermittently in milk for a number of years. In bulls, the structures targeted include seminal vesicles, ampullae, testicles and epididymes.

In affected herds, brucellosis can result in decreased fertility, reduced milk production, abortions in susceptible replacement animals and testicular degeneration in bulls. Abortion is a consequence of placentitis involving both cotyledons and intercotyledonary tissues. In bulls, necrotizing orchitis occasionally results in localized fibrotic lesions.

Although clinical signs are not specific for bovine brucellosis, abortions in first-calf heifers and replacement animals may suggest the presence of the disease. Clusters of MZN-positive coccobacilli may be evident in smears of cotyledons and MZN-positive organisms may also be detected in foetal abomasal contents and uterine discharges. Isolation and identification of *B. abortus* is confirmatory. Identification criteria for isolates include colonial appearance, MZN-positive organisms, bacterial cell agglutination with high-titred anti-

serum and rapid urease activity. Methods used for biotyping are outlined in Table 25.1. A range of serological tests, varying in sensitivity and specificity, is available for the identification of infected animals (Table 26.1). Molecular methods, such as PCR-based techniques for the detection of brucellae in tissues and fluids have been developed. National eradication schemes are based on the detection and slaughter of infected cattle. Three types of vaccines are used in cattle: strain 19 vaccine, adjuvanted 45/20 vaccine and RB51 vaccine. The strain 19 vaccine is administered to female calves up to five months of age. Vaccination of mature animals leads to persistent antibody titres. The 45/20 bacterin has been used in some national eradication schemes. Even when administered to adult animals, this vaccine does not induce persistent antibody titres. The RB51 strain is a stable, rough mutant which induces good protection against abortion without stimulating serological responses detectable in conventional brucellosis surveillance programmes.

Table 26.1 Tests used for the diagnosis of bovine brucellosis using milk or serum.

Test	Comments
Brucella milk ring test	Conducted on bulk milk samples for monitoring infections in dairy herds. Sensitive but may not be reliable in large herds
Rose-Bengal plate test	Useful screening test. Antigen suspension is adjusted to pH 3.6, allowing agglutination by IgG1 antibodies. Qualitative test only, positive results require confirmation by CFT or ELISA
Complement-fixation test (CFT)	Widely accepted confirmatory test for individual animals
Indirect ELISA	Reliable screening and confirmatory test
Competitive ELISA (using monoclonal antibodies)	Recently developed test with high specificity; capable of detecting all immunoglobulin classes and can be used to differentiate infected animals from S19-vaccinated cattle
Serum agglutination test (SAT)	A tube agglutination test which lacks specificity and sensitivity; IgG1 antibodies may not be detected, leading to false-negative results
Antiglobulin test	Sensitive test for detecting non-agglutinating antibodies not detected by the SAT

Caprine and ovine brucellosis

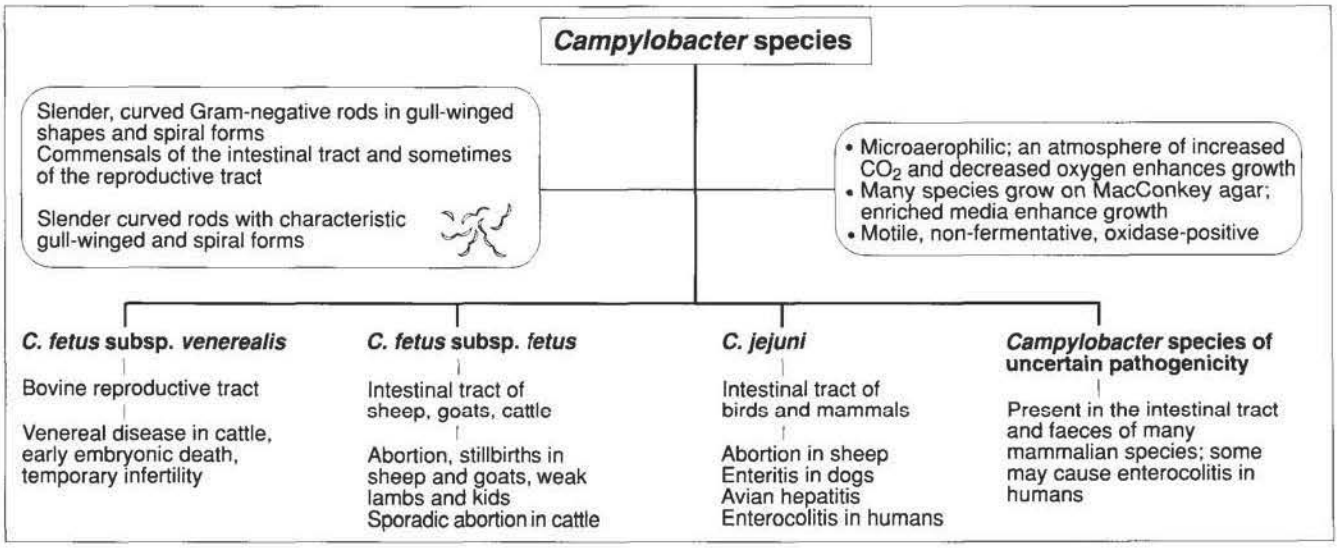
Brucellosis in goats and sheep, caused by *B. melitensis*, is most commonly encountered in countries around the Mediterranean littoral and in the Middle East, central Asia and parts of South America. Goats, in which the disease is more severe and protracted, tend to be more susceptible to infection than sheep. The clinical disease resembles brucellosis in cattle in many respects. Clinical features include high abortion rates in susceptible populations, orchitis in male animals, arthritis and hygromas. Infection resulting in abortion may not induce protective immunity.

Diagnosis is based on clinical signs, direct examination of MZN-stained smears of fluids or tissues, isolation and identification of *B. melitensis* and serological testing. In countries where the disease is exotic, a test and slaughter policy is usually implemented. The Rose-Bengal agglutination test and the complement fixation test are the most widely used serological methods for detecting infection with *B. melitensis*. The modified live *B. melitensis* Rev. 1 strain, administered by the subcutaneous or conjunctival routes, is used for vaccination of kids and lambs up to six months of age.

Brucellosis in humans

Humans are susceptible to infection with *B. abortus*, *B. suis*, *B. melitensis* and, rarely, *B. canis*. Transmission to humans occurs through contact with secretions or excretions of infected animals. Routes of entry include skin abrasions, inhalation and ingestion. Raw milk and dairy produce made with unpasteurized milk are important sources of infection. Brucellosis in humans, known as undulant fever, presents as fluctuating pyrexia, malaise, fatigue and muscle and joint pains. Abortion is not a feature of human infection. Osteomyelitis is the most common complication. Severe infection occurs with *B. melitensis* (Malta fever) and *B. suis* biotypes 1 and 2. Human infections due to *B. abortus* are moderately severe, whereas those caused by *B. canis* are usually mild.

27 *Campylobacter* species



Among Gram-negative bacteria, *Campylobacter* species have a number of distinguishing morphological features. They are slender, curved, motile Gram-negative rods with polar flagella. Daughter cells which remain joined have a characteristic gull-winged appearance and long spirals formed by joined cells also occur. These microaerophilic organisms grow best on enriched media in an atmosphere of increased CO_2 and decreased oxygen tension. Many *Campylobacter* species grow on MacConkey agar. They are non-fermentative and oxidase-positive. *Campylobacter* species are found in the intestinal and genital tracts of domestic animals and are widely distributed geographically. *Campylobacter jejuni* subspecies *jejuni* (referred to as *C. jejuni*) and *C. lari* colonize the intestines of birds which can result in faecal contamination of water courses and stored food. *Campylobacter fetus* subspecies *venerealis* appears to be adapted principally to bovine preputial mucosa. *Campylobacter* species are strictly microaerophilic, requiring an atmosphere of 5 to 10% CO_2 for growth. A selective enriched medium such as Skirrow agar is usually used for primary isolation. Differentiation of isolates is based on colonial morphology and certain cultural, biochemical and antibiotic-susceptibility characteristics. Some species such as *C. jejuni* grow optimally at 42°C. Differentiating characteristics of the main animal pathogens and some commonly isolated commensals are presented in Table 27.1.

The most important consequences of infections with organisms in this group are infertility in cattle due to *C. fetus* subspecies *venerealis* and abortion in ewes caused either by *C. fetus* subspecies *fetus* or by *C. jejuni*.

Bovine genital campylobacteriosis

Campylobacter fetus subspecies *venerealis*, the principal cause of bovine genital campylobacteriosis, is transmitted during coitus to susceptible cows by asymptomatic carrier bulls. The disease is characterized by temporary infertility associated with early embryonic death, return to oestrus at irregular periods and, occasionally, by sporadic abortion. About one-third of infected animals become carriers with the organisms persisting in the vagina of carrier cows. Extension of infection to the uterus with the development of endometritis and salpingitis can occur during the progestational phase of the oestrus cycle. The infertile period following uterine invasion can last for up to five months, after which natural immunity may develop. This natural immunity may last for up to four years. *Campylobacter fetus* subspecies *fetus*, an enteric organism acquired by ingestion, can cause sporadic abortions in cows.

Investigation of the breeding records and vaccination history of an affected herd may suggest campylobacteriosis. *Campylobacter* species can be detected by the fluorescent antibody technique in sheath washings from bulls or cervicovaginal mucus from cows. Isolation and identification of *C. fetus* subspecies *venerealis* from preputial or vaginal mucus is confirmatory. Dihydrostreptomycin, administered either systemically or topically, is used for treating bulls. Intrauterine administration of dihydrostreptomycin can be used therapeutically. Vaccination with bacterins in an oil emulsion adjuvant is used therapeutically and prophylactically in problem herds.

Ovine genital campylobacteriosis

Campylobacteriosis in ewes may be caused by either *C. fetus* subspecies *fetus* or *C. jejuni*. The disease, which is worldwide in distribution, is one of the most common causes of ovine abortion in some countries. *Campylobacter fetus* subspecies *fetus* is found in the faeces of cattle and sheep and *C. jejuni* may be present in the faeces of a wide range of birds and mammals. Trans-

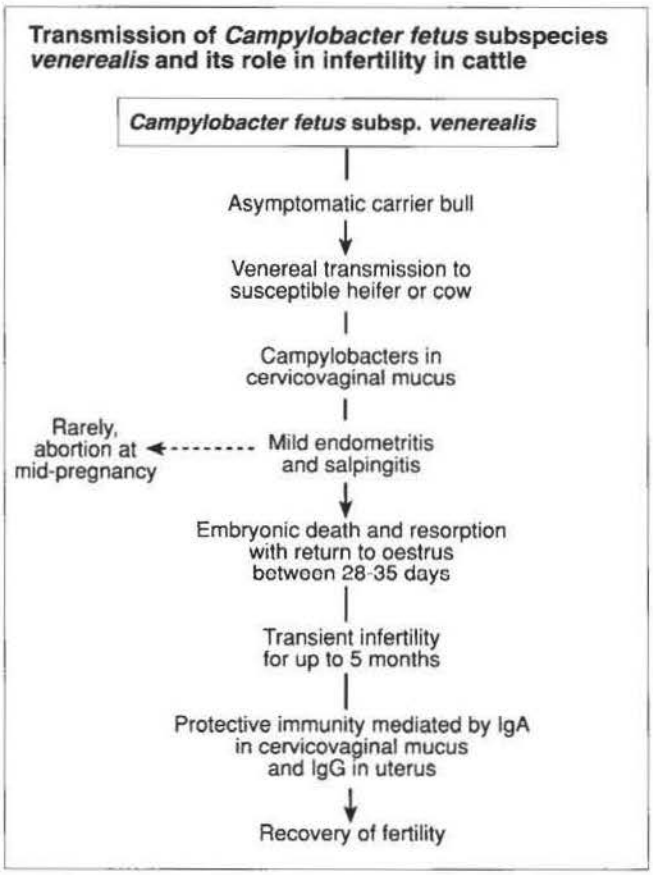
Table 27.1 Differentiating characteristics of *Campylobacter* species.

Campylobacter species	Catalase production	Growth at 25°C	Growth at 42°C	Growth in 1% glycine	Growth in 3.5% NaCl	Production of H_2S[a]	Susceptibility to Nalidixic acid[b]	Susceptibility to Cephalothin[b]
C. fetus subsp. *venerealis*	+	+	–	–	–	–	R	S
C. fetus subsp. *fetus*	+	+	–	+	–	+	V	S
C. jejuni subsp. *jejuni*	+	–	+	+	–	+	S	R
C. lari	+	–	+	+	–	+	R	R
C. coli	+	–	+	+	–	+	S	R

a lead acetate method of detection
b 30 µg discs
\+ most strains positive
– most strains negative
R resistant
S susceptible
V variable

mission of both of these organisms is by the faecal-oral route. During pregnancy, localization in the uterus of susceptible ewes may occur following bacteraemia. The subsequent necrotic placentitis may result in abortion late in pregnancy, stillborn lambs or weak lambs. Round, necrotic lesions up to 2 cm in diameter with pale raised rims and dark depressed centres are evident on the liver surface in some aborted lambs. Aborting ewes are major sources of infection for susceptible animals in a flock. Up to 20% of ewes in a susceptible flock may abort. Recovered ewes are immune for at least three years.

Typical hepatic lesions in aborted lambs are pathognomonic. Isolation and identification of *C. fetus* subspecies *fetus* or *C. jejuni* from foetal abomasal contents or birth fluids is confirmatory. Aborting ewes should be isolated and placentae and aborted foetuses promptly removed. The remainder of the flock should be moved to clean pasture. Vaccination of ewes with a *C. fetus* subspecies *fetus* bacterin, after confirmation of the disease in a flock, is reported to reduce the number of abortions.



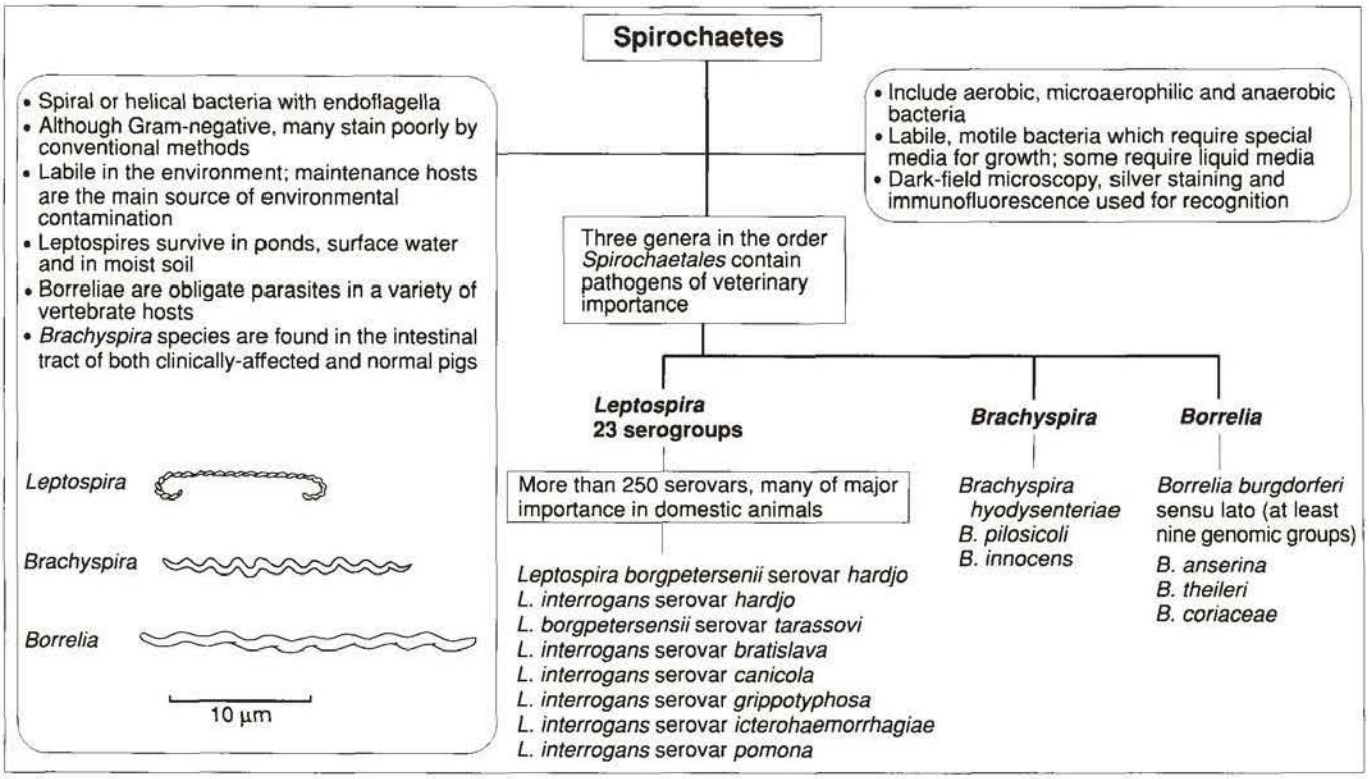
Intestinal campylobacteriosis in dogs

Diarrhoea in dogs and other domestic animals has been attributed to infection with *Campylobacter* species, particularly *C. jejuni*. Confirmation is difficult because healthy animals may shed *Campylobacter* species in their faeces. However, the presence of large numbers of campylobacter-like organisms in dilute carbol fuchsin-stained faecal smears or rectal scrapings from dogs with diarrhoea may be indicative of infection. *Campylobacter* species may contribute to the severity of enteric disease in dogs infected with other enteropathogens such as enteric viruses, *Giardia* species and helminths. Dogs shedding *C. jejuni* are a potential source of human infection.

Intestinal campylobacteriosis in humans

Campylobacter jejuni is the main cause of human intestinal campylobacteriosis. *Campylobacter coli* and *C. lari* are sometimes implicated. These zoonotic infections are usually foodborne and poultry meat is the main source of human infection. Fever, abdominal pain and diarrhoea, sometimes with blood, are the most common manifestations of this enteric infection.

28 Spirochaetes 1



Pathogens in the family *Leptospiraceae* belong to the genus *Leptospira*. The genera *Borrelia*, *Brachyspira* and *Treponema* in the family *Spirochaetaceae* contain significant animal and human pathogens. There are some non-pathogenic genera in each family. Pathogenic spirochaetes are difficult to culture. Many require specialized media and some require liquid media.

Leptospira species

Members of this species (leptospires) are motile helical bacteria with hook-shaped ends. Although cytochemically Gram-negative, they do not stain well with conventional bacteriological dyes and dark-field microscopy is used for their detection in fluids or liquid media. Silver impregnation and immunological staining techniques are used to demonstrate leptospires in tissues. Leptospirosis, which can affect all domestic animals and humans, ranges in severity from mild infections of the urinary or genital systems to serious systemic disease.

Leptospires can survive in ponds, rivers, surface waters, moist soil and mud when environmental temperatures are mild. Pathogenic leptospires can persist in the renal tubules or in the genital tract of carrier animals. These fragile organisms are transmitted most effectively by direct contact. Leptospiral species (genospecies) are classified by DNA homology and, within each species, various serovars are recognized on the basis of serological reactions. At present, more than 250 serovars in 23 serogroups are defined. Serovars with antigens in common belong to the same serogroup.

Although leptospires are found worldwide, some serovars appear to have a limited geographical distribution. In addition, most serovars are associated with a particular species, their

Structural features of a typical spirochaete (A) and their relationships in cross section (B).

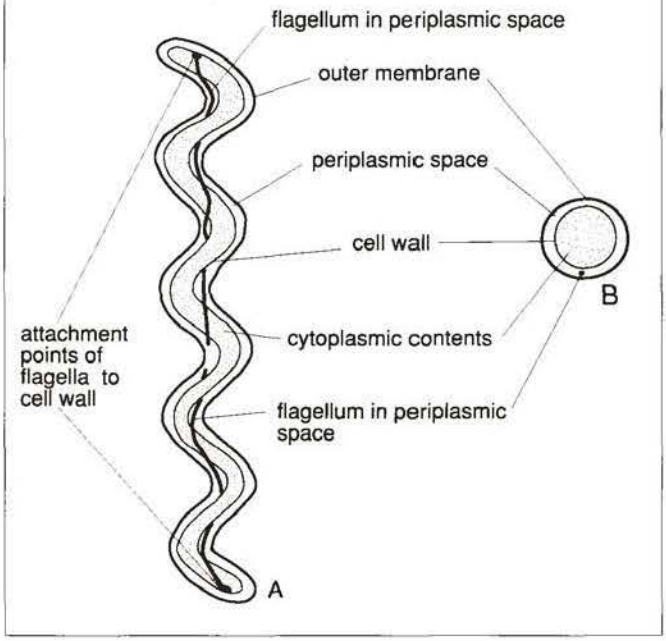


Table 28.1 Serovars of *Leptospira* which cause leptospirosis in domestic animals.

Serovar	Hosts	Clinical conditions
Leptospira borgpetersenii serovar *hardjo* *L. interrogans* serovar *hardjo*	Cattle, sheep	Abortions, stillbirths, agalactia
	Humans	Influenza-like illness; occasionally liver or kidney disease
L. borgpetersenii serovar *tarassovi*	Pigs	Reproductive failure, abortions, stillbirths
L. interrogans serovar *bratislava*	Pigs, horses, dogs	Reproductive failure, abortions, stillbirths
L. interrogans serovar *canicola*	Dogs	Acute nephritis in pups. Chronic renal disease in adult animals
	Pigs	Abortions and stillbirths. Renal disease in young pigs
L. interrogans serovar *grippotyphosa*	Cattle, pigs, dogs	Septicaemic disease in young animals; abortion
L. interrogans serovar *icterohaemorrhagiae*	Cattle, sheep, pigs	Acute septicaemic disease in calves, piglets and lambs; abortions
	Dogs, humans	Peracute haemorrhagic disease; acute hepatitis with jaundice
L. interrogans serovar *pomona*	Cattle, sheep	Acute haemolytic disease in calves and lambs; abortions
	Pigs	Reproductive failure; septicaemia in piglets
	Horses	Abortions, periodic ophthalmia

maintenance host. The pathogenicity of leptospires relates to the virulence of the infecting serovar and the susceptibility of the host species. Leptospires invade tissues through moist, softened skin or through mucous membranes. They spread through the body via the blood stream but, following the appearance of antibodies about 10 days after infection, they are cleared from the circulation. Some organisms may evade the immune response and persist in the body, principally in the renal tubules and also in the uterus, eye or meninges. In susceptible animals, damage to red cell membranes and to endothelial cells, along with hepatocellular injury produces haemoglobinuria and haemorrhage, associated with acute leptospirosis.

Clinical signs, together with a history suggestive of clinical exposure to contaminated urine, may suggest acute leptospirosis. Leptospires may be isolated from the blood during the first ten days of infection and from the urine approximately two weeks after initial infection, by culture in liquid medium at 30°C or by animal inoculation. Isolates should be identified using DNA profiles and serology. Fluorescent antibody procedures are often used for the demonstration of leptospires in tissues. Silver impregnation techniques can also be used for demonstration of leptospires in tissues. The microscopic agglutination test, using live culture growth in a liquid medium, is a common serological test for the diagnosis of leptospirosis. A number of ELISA tests have also been developed. The disease conditions associated with leptospiral infections in domestic animals are presented in Table 28.1.

Leptospirosis in cattle and sheep

Cattle are maintenance hosts for *L. borgpetersenii* serovar *hardjo* and this serovar may also be host-adapted for sheep. *Leptospira interrogans* serovar *hardjo* is also host-adapted for cattle. Susceptible replacement heifers, reared separately and introduced into an infected dairy herd for the first time at calving, may develop acute disease with pyrexia and agalactia affecting all quarters. Infection may also result in abortion and stillbirths. Serovars incorporated into vaccines should be those associated with disease in a particular region.

Leptospirosis in horses

Clinical disease most often results from incidental infection with serovar *pomona*. Signs include abortion in mares and renal disease in young horses. An immune-mediated anterior uveitis (periodic ophthalmia, 'moon blindness') may be a manifestation of chronic leptospirosis in horses.

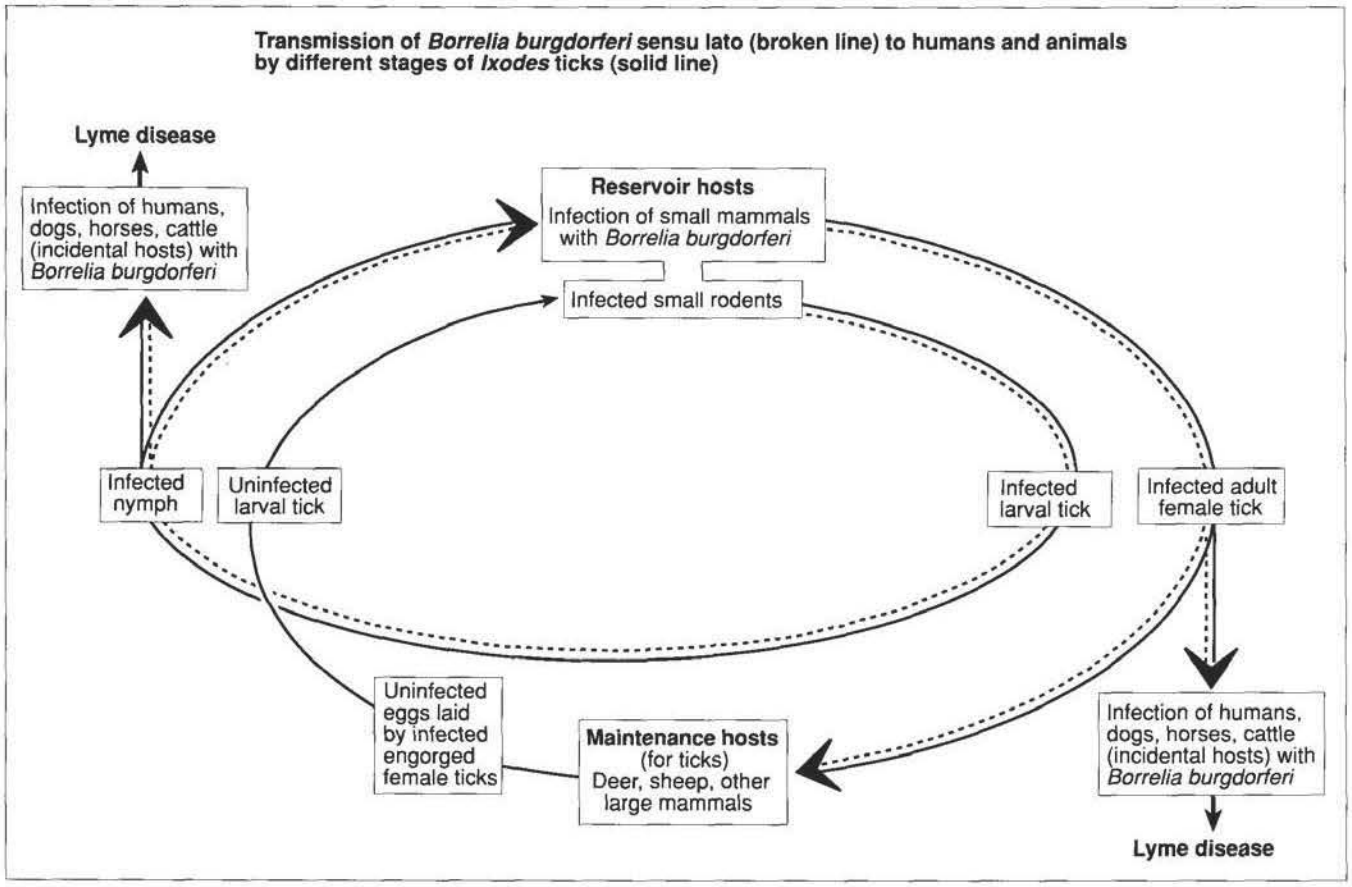
Leptospirosis in pigs

Acute leptospirosis in pigs is usually caused by rodent-adapted serovars such as *icterohaemorrhagiae* and *copenhagenii*. These serovars cause serious, sometimes fatal, disease in young pigs. In many parts of the world, the principal host-adapted serovar is *pomona*. Infection can result in reproductive failure including abortions and stillbirths.

Leptospirosis in dogs and cats

The serovars associated with leptospirosis in dogs and cats are *canicola* and *icterohaemorrhagiae*. Serovar *canicola*, which is host-adapted for dogs, causes severe renal disease in pups. Incidental canine infections, usually caused by *icterohaemorrhagiae*, are characterized by acute haemorrhagic disease or subacute hepatic and renal failure. Clinical leptospirosis is uncommon in cats.

29 Spirochaetes 2



Borrelia species

Borreliae, which are longer and wider than other spirochaetes, have a similar helical shape. Although these spirochaetes can cause disease in animals and humans, subclinical infections are also common. Borreliae are transmitted by arthropod vectors. These spirochaetes are obligate parasites in a variety of vertebrate hosts and they depend on vetebrate reservoir hosts and arthropod vectors for long-term survival. Borreliae can be differentiated from other spirochaetes by their morphology, by the low guanine and cytosine content of their genomic DNA and by ecological, cultural and biochemical characteristics. Identification of *Borrelia* species depends mainly on genetic analysis. At least nine genomic groups of *B. burgdorferi* sensu lato have been identified. The species of particular veterinary importance are *B. burgdorferi* sensu lato, the cause of Lyme disease in animals and humans, and *B. anserina* which causes avian borreliosis (Table 29.1).

Lyme disease

This condition was first identified in 1975 following investigation of a cluster of arthritis cases in children near the town of Old Lyme, Connecticut. The causative agent, a spirochaete, was named *Borrelia burgdorferi*. Several genospecies of *B. burgdorferi* have subsequently been identified in the USA and Europe. Lyme disease has been reported in humans, dogs, horses and cattle, and infection has been documented in sheep.

Ticks are the only competent vectors of *B. burgdorferi* sensu lato. Infection is usually acquired by larval stages of ticks feeding on small rodents. The spirochaetes persist through nymphal and adult stages of ticks which transmit infection while feeding. The persistence of these pathogenic bacteria in a region is dependent on the presence of suitable reservoir hosts for borreliae and maintenance hosts for ticks. The most common tick vector for *B. burgdorferi* sensu lato in Europe is *Ixodes ricinus*. In the USA, different *Ixodes* species act as vectors: *I. scapularis* in central and eastern regions, *I. pacificus* on the west coast. After entering the bloodstream of a susceptible host, borreliae multiply and are disseminated throughout the body. Organisms can be demonstrated in joints, brain, nerves, eyes and heart.

Most infections are subclinical and serological surveys demonstrate that exposure is common in both animal and human populations in endemic areas. The clinical manifestations of Lyme disease relate mainly to the sites of localization of the organisms. Clinical disease is reported frequently in dogs.

Table 29.1 Tick vectors and natural hosts of *Borrelia* species and associated clinical conditions.

Species	Vector	Host	Clinical conditions
B. burgdorferi sensu lato	*Ixodes* species	Rodents	Arthritic, neurological and cardiac disease in dogs and occasionally in horses, cattle, sheep and humans
B. anserina	*Argas* species	Birds	Fever, weight loss and anaemia in domestic poultry
B. theileri	Many species of ticks	Cattle, sheep, horses	Mild, febrile disease with anaemia
B. coriaceae	*Ornithodoros* species	Cattle, deer	Associated with epizootic bovine abortion in USA

Signs include fever, lethargy, arthritis and evidence of cardiac, renal or neurological disturbance. The clinical signs in horses are similar to those in dogs. In cattle and sheep, lameness has been reported. Laboratory confirmation of Lyme disease may prove difficult because the spirochaetes may be present in low numbers in specimens from clinically affected animals. A history of exposure to tick infestation in an endemic area in association with characteristic clinical signs may suggest Lyme disease. Rising antibody titres to *B. burgdorferi* sensu lato along with typical clinical signs are indicative of disease. ELISA and immunofluorescence assays are used for antibody detection. Culture of borreliae from clinically affected animals is confirmatory. Low numbers of borreliae can be detected in samples by PCR techniques.

Acaricidal sprays, baths or dips should be used to control tick infestation. Where feasible, tick habitats such as rough brush and scrub should be cleared. A number of vaccines, including whole-cell bacterins and a recombinant subunit vaccine, are commercially available for use in dogs.

Lyme disease is an important tick-borne infection of humans. Infection is often acquired by walking in endemic areas during periods of tick activity. Clinical signs include skin rash at site of tick attachment followed by arthritis, muscle pains, and cardiac and neurological abnormalities.

Avian spirochaetosis

This acute disease of birds, caused by *Borrelia anserina*, can result in significant economic loss in flocks in tropical and subtropical regions. Chickens, turkeys, pheasants, ducks and geese are susceptible to infection. Soft ticks of the genus *Argas* frequently transmit the disease. The borreliae survive trans-stadial moulting in ticks and can be transmitted transovarially between tick generations. Outbreaks of avian spirochaetosis coincide with periods of peak tick activity during warm, humid seasons. The disease is characterized by fever, marked anaemia and weight loss. Paralysis may develop as the disease progresses. Immunity, which follows recovery, is serotype-specific. Diagnosis can be confirmed by demonstration of the spirochaetes in buffy coat smears using dark-field microscopy. Giemsa-stained smears or silver impregnation techniques can be used to demonstrate the borreliae in tissues. Blood or tissue smears can be examined using immunofluorescence. Inactivated vaccines and tick eradication are the main control measures.

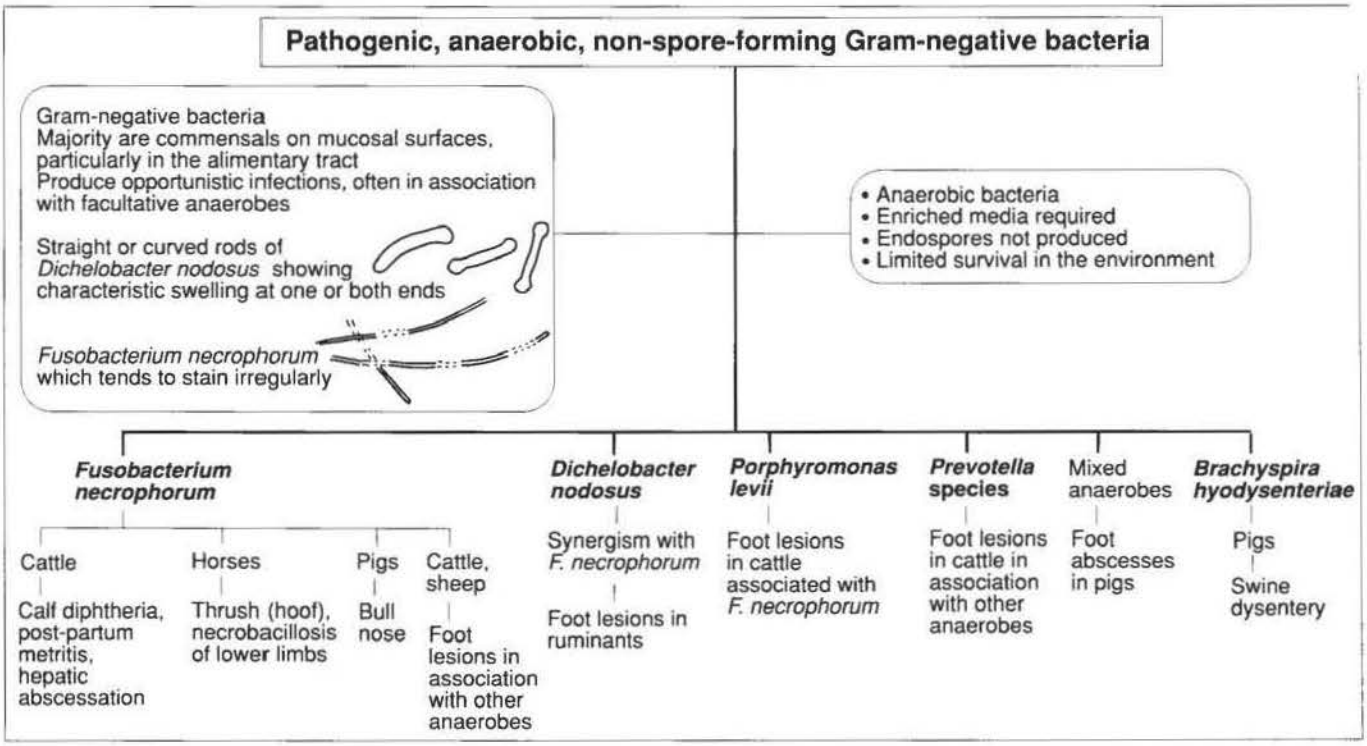
Brachyspira and *Serpulina* species

Five genospecies of intestinal spirochaetes, *Brachyspira hyodysenteriae*, *B. pilosicoli*, *B. innocens*, *Serpulina intermedia* and *S. murdochii* have been isolated from pigs. These anaerobic spirochaetes can be differentiated by their pattern of haemolysis on blood agar, hippurate hydrolysis and by restriction endonuclease analysis. Pathogenic *Brachyspira* species are found in the intestinal tract of both clinically affected and normal pigs. Carrier pigs can shed *B. hyodysenteriae* for up to three months and carrier pigs are the principal source of infection for healthy pigs. Colonization is enhanced by factors in mucus with chemotactic activity for *B. hyodysenteriae*. Haemolytic activity, demonstrated *in vitro*, correlates with pathogenicity.

Infections with *Brachyspira* species are of importance in pigs. *Brachyspira hyodysenteriae*, the cause of swine dysentery, and *B. pilosicoli*, the cause of porcine intestinal spirochaetosis, are recognized pathogens. Pigs acquire infection through exposure to contaminated faeces. Rodents and flies may act as transport hosts for the spirochaetes. *Brachyspira hyodysenteriae* can persist for several weeks in moist faeces. Infection with *B. hyodysenteriae* causes dysentery which is often encountered in weaned pigs from six to twelve weeks of age. Affected pigs lose condition and become emaciated. Appetite decreases and thirst may be evident. During recovery, there may be large amounts of mucus in the faeces. Although mortality is low, reduced weight gains due to poor food conversion causes major economic loss. The clinical signs in porcine intestinal spirochaetosis, caused by *B. pilosicoli*, are similar to those of swine dysentery but are less severe. Diarrhoea contains mucus rather than blood.

History, clinical signs and gross lesions may indicate swine dysentery. Blood agar with added antibiotics is used for the culture of *Brachyspira* species. Cultures are incubated anaerobically at 42°C for at least three days. Definitive identification can be made using immunofluorescence, DNA probes or biochemical tests. Medication of drinking water is a useful method of treatment. Depopulation, thorough cleaning, disinfection of premises and strict rodent control are required for eradication of disease.

30 Pathogenic anaerobic non-spore-forming Gram-negative bacteria

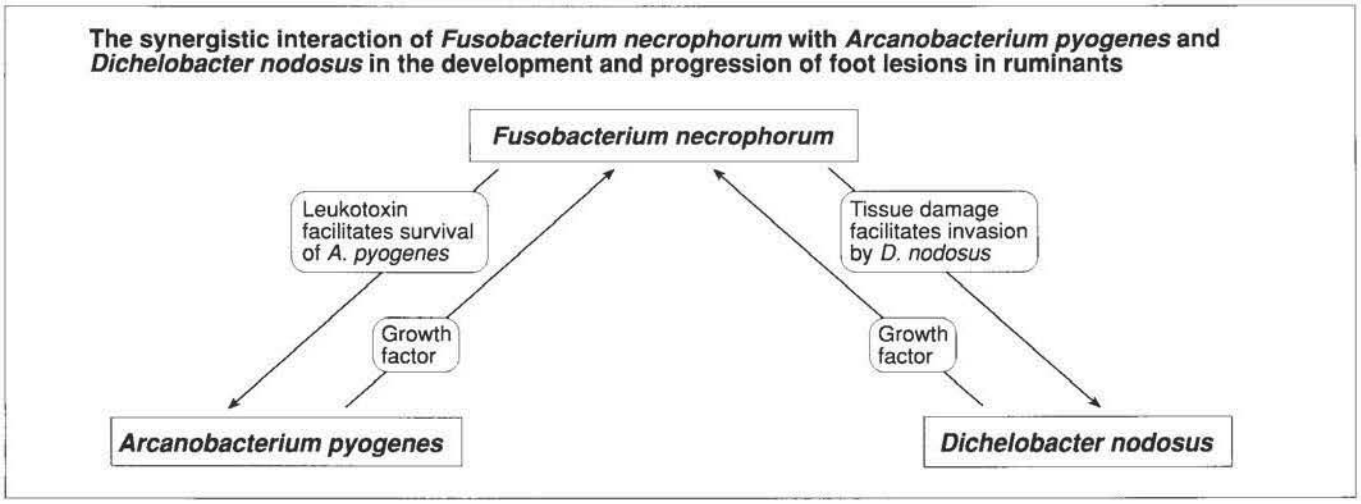


Many non-spore-forming, anaerobic, Gram-negative bacteria cause opportunistic mixed infections, often in association with facultative anaerobes. Synergistic interactions between the organisms in these mixed infections are common. Non-spore-forming, Gram-negative anaerobes are often found on mucous membranes, particularly in the digestive tract of animals and humans. These bacteria are differentiated on the basis of bacterial morphology, colonial appearance, antibiotic susceptibility testing and fatty acid production. Anaerobic jars with an atmosphere of hydrogen and 10% CO_2 are used for incubating cultures at 37°C for up to seven days. Specimens should be processed promptly after collection. Colonies of Gram-negative anaerobes usually have a foetid odour due to volatile fatty acid production.

Non-spore-forming Gram-negative anaerobes usually exert their pathogenic effects when anatomical barriers are breached allowing invasion of underlying tissues. They replicate only at low or negative reduction potentials (*E*h). Tissue trauma and necrosis, followed by multiplication of facultatively anaerobic bacteria, can lower *E*h levels to a range suitable for the proliferation of non-spore-forming anaerobes. Most infections involving these organisms are mixed. Two or more bacterial species, interacting synergistically, may produce lesions which individual organisms cannot. The synergism between *Arcanobacterium pyogenes* and *Fusobacterium necrophorum* relates to growth factor production by the latter. Synergism between *F. necrophorum* and *Dichelobacter nodosus* is also important in the pathogenesis of ruminant pedal lesions. *Fusobacterium necrophorum* is considered to be the primary pathogen in a number of disease conditions in farm animals (Table 30.1). Mixed bacterial infections are commonly implicated in foot lesions in domestic ruminants and pigs (Table 30.2). Mixed infections with non-spore-forming anaerobes are also present in

Table 30.1 Disease conditions of farm animals in which *Fusobacterium necrophorum* plays a primary role.

Species	Disease condition	Predisposing factors
Cattle	Calf diphtheria	Rough feed producing mucosal damage
	Post-partum metritis	Dystocia
	Hepatic abscessation	Sudden dietary change leading to acidosis and rumenitis
	Black spot of teat	Trauma to region adjacent to teat sphincter
Horses	Thrush (hoof)	Poor hygiene and wet housing conditions
	Necrobacillosis of lower limbs	Poor hygiene
Pigs	Bull nose	Trauma to nasal mucosa



aspiration pneumonias and in bovine traumatic reticuloperitonitis and pericarditis.

Calf diphtheria

This condition usually presents as necrotic pharyngitis or laryngitis in calves under three months of age. *Fusobacterium necrophorum* can enter through abrasions in the mucosa of the pharynx or larynx often caused by ingestion of coarse feed. Clinical signs include fever, depression, anorexia, excessive salivation, respiratory distress and a foul smell from the mouth. Untreated calves may develop a fatal necrotizing pneumonia.

Bovine liver abscess

Hepatic abscessation in cattle, secondary to rumenitis, is encountered most commonly in feedlot animals. The feeding of rations high in carbohydrates and the resulting rapid intraruminal fermentation can lead to the development of ulcers. *Fusobacterium necrophorum* together with other anaerobes and *Arcanobacterium pyogenes* invade the tissues, and occasional emboli which reach the liver via the portal vein initiate abscess formation. Affected cattle rarely show clinical signs and lesions are usually detected at slaughter.

Necrotic rhinitis in pigs

This sporadic condition, primarily affecting young pigs, is characterized by suppuration and necrosis of the snout as a result of infection with *F. necrophorum*, often in association with other anaerobes. Those organisms usually enter through abrasions in the nasal mucosa. Signs include swelling of the face, sneezing and a foul-smelling nasal discharge. In chronic infections, involvement of the nasal and facial bones can result in permanent deformity ('bull nose').

Thrush of the hoof

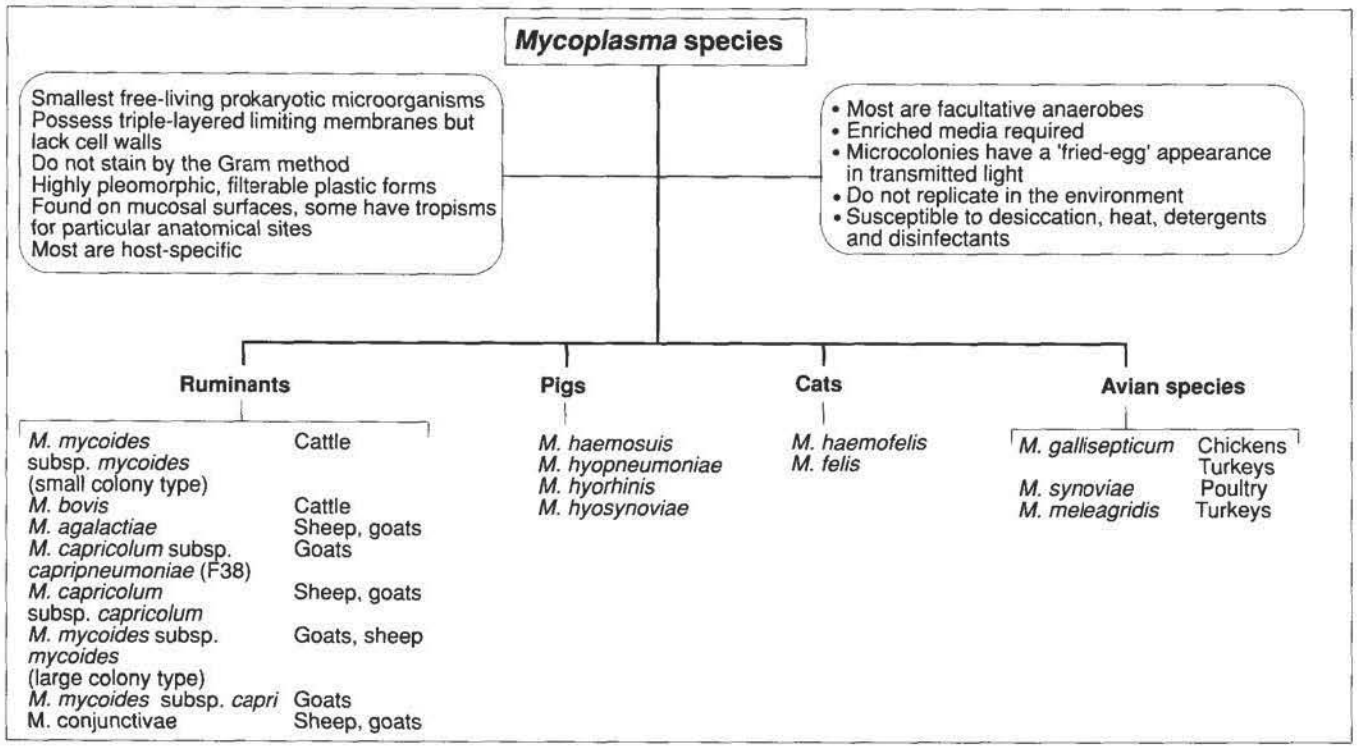
This necrotic condition of the horses hoof is associated with poor hygiene, wet conditions and lack of regular cleaning of the hooves. Infection with *F. necrophorum*, secondary to hoof damage, results in localized inflammation. Thrush, which commonly affects the hind feet, is characterized by a foul-smelling discharge in the sulci, close to the frog. Dry, clean stabling, regular attention to the hooves and exercise, promote frog regeneration.

Table 30.2 Foot conditions in farm animals associated with mixed infections including anaerobic non-spore-forming bacteria.

Species	Disease condition	Bacteria implicated
Sheep	Interdigital dermatitis	*Fusobacterium necrophorum* *Dichelobacter nodosus* (benign strains)
	Heel abscess and lamellar suppuration	Mixed anaerobic flora including *Arcanobacterium pyogenes*[a] *F. necrophorum*, and others
	Footrot	*Dichelobacter nodosus* *Fusobacterium necrophorum* *Arcanobacterium pyogenes*[a] Unidentified spirochaete
Cattle	Interdigital necrobacillosis (Foul-in-the-foot)	*Fusobacterium necrophorum* *Porphyromonas levii*
	Interdigital dermatitis	*Dichelobacter nodosus* *Fusobacterium necrophorum* *Prevotella* species Spirochaetes?
Pigs	Foot abscess in young pigs and bush foot (lamellar suppuration) in older animals	Mixed anaerobes

a facultatively anaerobic

31 Mycoplasmas



Mycoplasmas, the smallest prokaryotic cells capable of self-replication, are pleomorphic organisms. Because they cannot synthesize peptidoglycan or its precursors, they do not possess rigid cell walls but have flexible triple-layered outer membranes. They are resistant to antibiotics such as penicillin which interfere with the synthesis of bacterial cell walls. They require enriched media for growth and, although most mycoplasmas are facultative anaerobes, some grow optimally in an atmosphere of 5% to 10% CO_2. Characteristically, microcolonies have an umbonate appearance when illuminated obliquely and a 'fried-egg' appearance in transmitted light.

Mycoplasmas are found on mucosal surfaces of the conjunctiva, nasal cavity, oropharynx, intestinal and genital tracts of animals and humans. In general, they are host-specific and survive for short periods in the environment. The genera *Mycoplasma* and *Ureaplasma* contain animal pathogens. The major diseases associated with infection by *Mycoplasma* species are summarized in Table 31.1.

Mycoplasmas are differentiated by their host specificity, colonial morphology, requirement for cholesterol and biochemical reactivity. For growth, these organisms require enriched media containing animal protein, a sterol component or adenine dinucleotide. Immunological tests, using specific antisera produced against each pathogenic species, are required for definitive identification. Growth inhibition tests are used for diagnosis. Fluorescent antibody staining of individual microcolonies can also be used for identification. Rapid plate agglutination tests are used for screening poultry flocks and for the field diagnosis of contagious bovine pleuropneumonia.

Mycoplasmas adhere to host cells, an attribute essential for pathogenicity. This close contact, which facilitates toxic damage to host cells by soluble factors produced by the

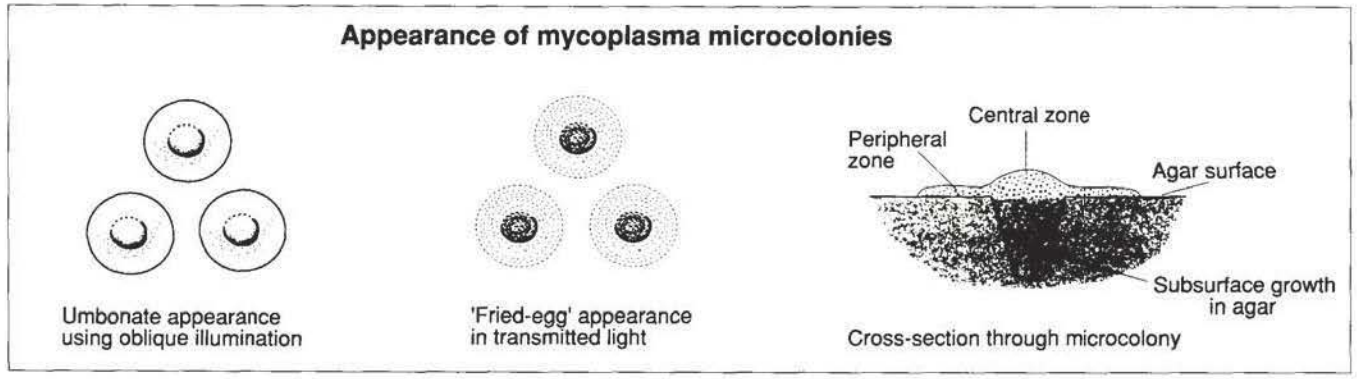


Table 31.1 Mycoplasma species of veterinary significance, the disease conditions which they cause and their geographical distribution.

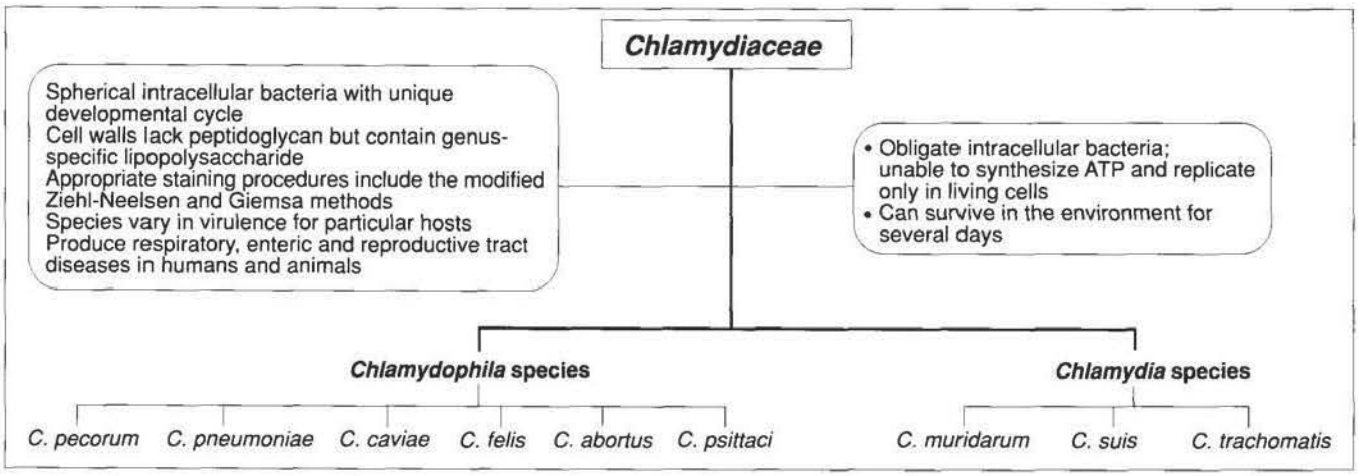
Mycoplasma species	Hosts	Disease conditions	Geographical distribution
M. mycoides subsp. *mycoides* (small colony type)	Cattle	Contagious bovine pleuropneumonia	Endemic in parts of Africa, Middle East, Asia; sporadic outbreaks in some European countries
M. bovis	Cattle	Mastitis, pneumonia, arthritis	Worldwide
M. agalactiae	Sheep, goats	Contagious agalactia	Parts of Europe, northern Africa, western Asia
M. capricolum subsp. *capripneumoniae* (F38)	Goats	Contagious caprine pleuropneumonia	Northern and eastern Africa, Turkey
M. capricolum subsp. *capricolum*	Sheep, goats	Septicaemia, mastitis, polyarthritis, pneumonia	Africa, Europe, Australia, USA
M. mycoides subsp. *mycoides* (large colony type)	Goats, sheep	Pleuropneumonia, mastitis, septicaemia, polyarthritis	Middle East, North America, India, parts of Europe
M. mycoides subsp. *capri*	Goats	Septicaemia, pleuropneumonia, arthritis, mastitis	Parts of Asia, Africa, Europe, Australia
M. hyopneumoniae	Pigs	Enzootic pneumonia	Worldwide
M. hyorhinis	Pigs (3-10 weeks of age)	Polyserositis	Worldwide
M. hyosynoviae	Pigs (10-30 weeks of age)	Polyarthritis	Worldwide
M. haemosuis (formerly *Eperythrozoon suis*)	Pigs	Infection often subclinical but may cause anaemia and jaundice. Transmitted by biting arthropods	Worldwide
M. gallisepticum	Chickens Turkeys	Chronic respiratory disease Infectious sinusitis	Worldwide
M. synoviae	Poultry	Infectious synovitis	Worldwide
M. meleagridis	Turkeys	Airsacculitis, bone deformities, reduced hatchability and growth rate	Worldwide
M. haemofelis (formerly *Haemobartonella felis*)	Cats	Feline infectious anaemia. Transmitted through bite-wounds or biting arthropods	Worldwide

pathogen, often occurs on mucosal surfaces. Factors such as extremes of age, stress and intercurrent infections may predispose to tissue invasion. In addition, mycoplasmas may exacerbate disease initiated by other pathogens, particularly in the respiratory tract. Mycoplasmal infections cause respiratory diseases of major economic importance in farm animals, especially in ruminants, pigs and poultry.

Contagious bovine pleuropneumonia, a severe contagious disease of cattle, is caused by *M. mycoides* subspecies *mycoides*. The disease, which is transmitted by aerosols, requires close contact with clinically affected animals or asymptomatic carriers. Although spread of infection may be slow, the mortality rate may be high. The acute form of the disease is characterized by sudden onset of high fever, anorexia, depression, accelerated respiration and coughing. At postmortem, pneumonic lungs have a marbled appearance. The disease can be confirmed by isolation, and definitive identification of the pathogen by serology and by molecular techniques. In countries where the disease is exotic, slaughter of affected and in-contact cattle is mandatory. In endemic regions, control strategies are based on prohibiting movement of suspect animals, mandatory quarantine and the elimination of carrier animals by serological testing and slaughter. Annual vaccination with attenuated vaccines is carried out in endemic areas.

Enzootic pneumonia of pigs, caused by *M. hyopneumoniae*, is an economically important disease which occurs worldwide in intensively reared pigs. Poor ventilation, overcrowding and temperature fluctuations may precipitate an outbreak. Clinical, epidemiological and pathological findings are usually indicative of the presence of the condition. Although antimicrobial drugs such as tylosin tartarate or tiamulin are used therapeutically, prevention and control are primarily based on the development of specific-pathogen-free herds.

32 *Chlamydia* and *Chlamydophila* species

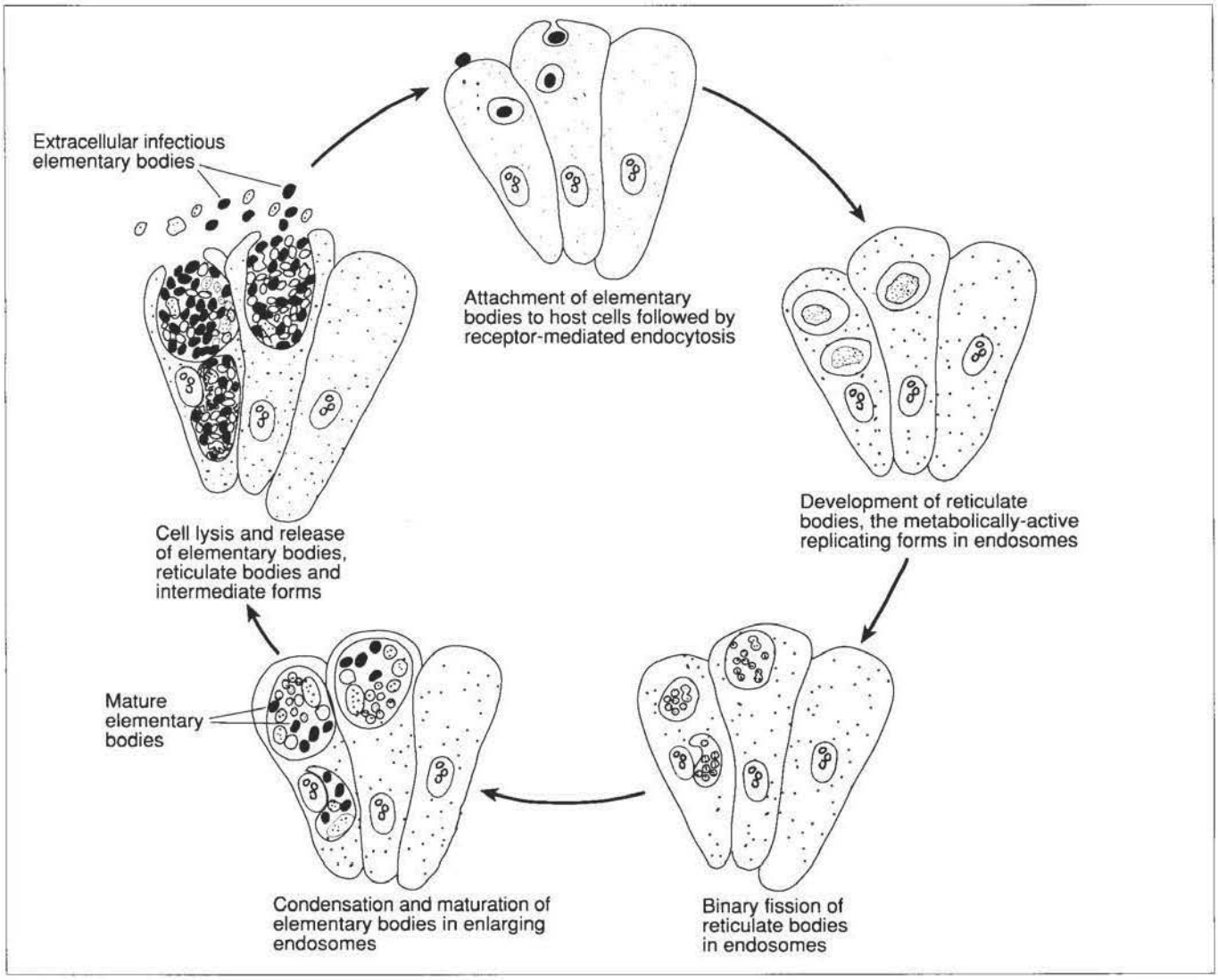


Chlamydiae are obligate intracellular bacteria with an unusual developmental cycle during which unique infectious forms are produced. They replicate within cytoplasmic vacuoles in host cells. On account of their apparent inability to generate ATP, with resultant dependence on host cell metabolism, they have been termed 'energy parasites'. The family *Chlamydiaceae* belongs to the order *Chlamydiales*. Currently two genera, *Chlamydia* and *Chlamydophila*, and nine species are described based on nucleic acid sequencing studies of the 16S and 23S rRNA genes.

In the developmental cycle of chlamydiae, infectious and reproductive forms are morphologically distinct. Infectious extracellular forms, called elementary bodies (EBs) are small (200 to 300 nm), metabolically inert and osmotically stable. Each EB is surrounded by a conventional bacterial cytoplasmic membrane, a periplasmic space and an outer envelope containing lipopolysaccharide. The periplasmic space does not contain a detectable peptidoglycan layer and the EB relies on disulphide cross-linked envelope proteins for osmotic stability. Elementary bodies enter host cells by receptor-mediated endocytosis. Acidification of the endosome and fusion with lysosomes are prevented by mechanisms which are not fully understood. A process of structural reorganization within the pathogen, of several hours duration, results in the conversion of an EB into a reticulate body (RB). The RB, about 1 μm in diameter, is metabolically active, osmotically fragile and replicates by binary fission within the endosome. The endosome and its contents, when stained, are called an inclusion. About 20 hours after infection, the developmental cycle becomes asynchronous with some RBs continuing to divide while others condense and mature to form EBs. In general, replication continues for up to 72 hours after infection when the host cell lyses releasing several hundred bodies which include EBs, RBs and intermediate forms. Chlamydial replication may be delayed in the presence of gamma interferon or penicillin or when the availability

Table 32.1 Chlamydial infections of veterinary and zoonotic importance.

Pathogen	Hosts	Clinical conditions
Chlamydophila psittaci	Birds	Pneumonia and airsacculitis Intestinal infection and diarrhoea Conjunctivitis Pericarditis Encephalitis
	Humans	Psittacosis/ornithosis
C. abortus	Sheep	Enzootic abortion of ewes (EAE)
	Goats	Chlamydial abortion
	Cattle	Chlamydial abortion
	Pigs	Chlamydial abortion
	Humans	Abortion
C. felis	Cats	Conjunctivitis (feline pneumonitis)
C. caviae	Guinea-pigs	Guinea-pig inclusion conjunctivitis
C. pecorum	Sheep	Intestinal infection Conjunctivitis Polyarthritis
	Cattle	Sporadic bovine encephalomyelitis Polyarthritis Metritis
Chlamydia suis	Pigs	Intestinal infection
C. muridarum	Mice	Respiratory infection



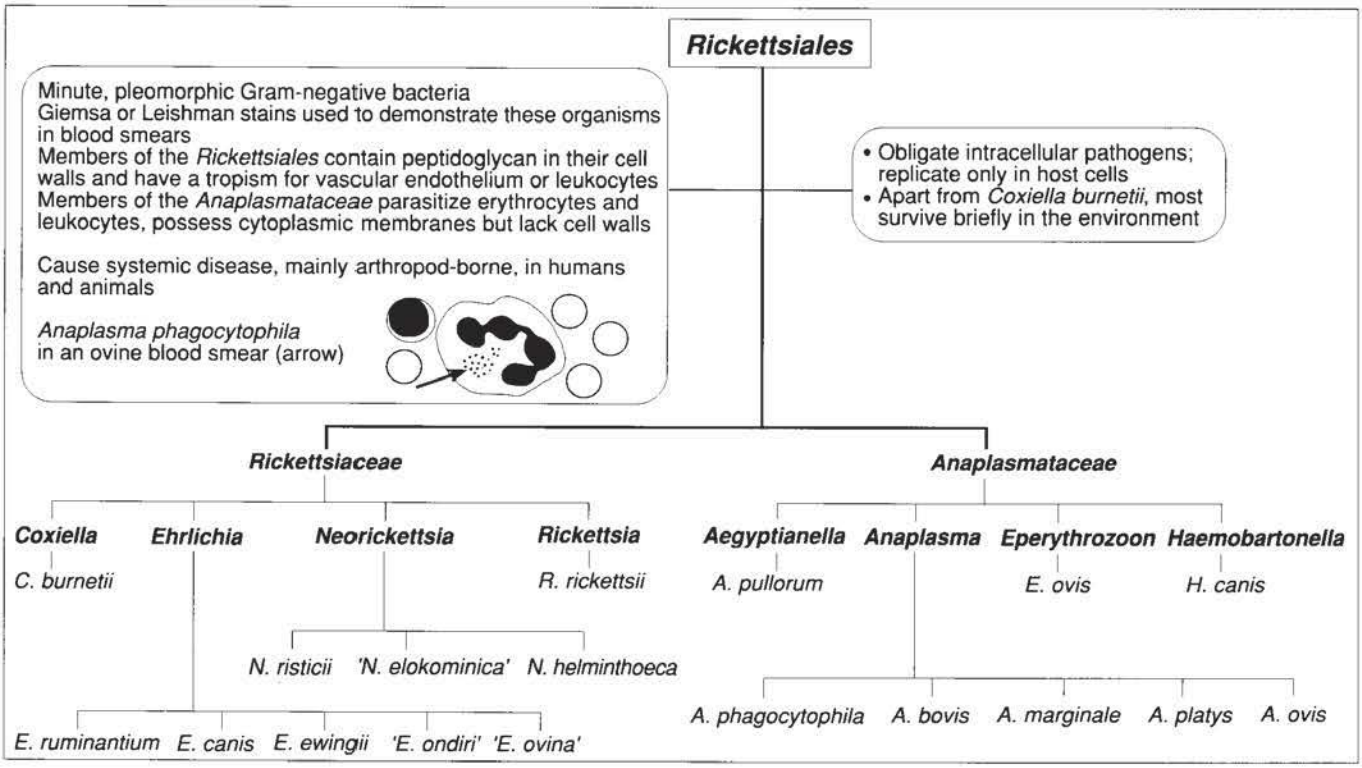
of tryptophan or cysteine is limited, resulting in persistent infection.

Chlamydiae infect over 130 species of birds and a large number of mammalian species including humans (Table 32.1). In recent years isolations have also been made from invertebrate species. Chlamydial species are usually associated with specific diseases in particular hosts. Both the severity and the type of disease produced by chlamydiae are highly variable, ranging from clinically inapparent infections and local infections of epithelial surfaces to severe systemic infections. Diseases associated with chlamydial infections include conjunctivitis, arthritis, abortion, urethritis, enteritis, pneumonia and encephalomyelitis. Clinical signs and their severity are influenced by factors related to both host and pathogen, and one type of clinical presentation usually predominates in outbreaks of disease. Infection with *C. pecorum* is associated with conjunctivitis, arthritis and inapparent intestinal infection. The type of clinical presentation relates to the route of infection and the degree of exposure. Environmental factors and management practices can influence the prevalence of some chlamydial infections such as enzootic abortion in ewes, which tend to be more prevalent in intensively-managed lowland flocks.

The gastrointestinal tract appears to be the usual site of *Chlamydophila* species infection in animals. Intestinal infections are often subclinical and persistent. Faecal shedding of the organisms, which is typically prolonged, becomes intermittent with time. The EBs can survive in the environment for several days. In sheep, *C. abortus* is an important cause of abortion whereas infections with *C. pecorum* are frequently inapparent. Inter-species transmission is uncommon. When it occurs, the outcome of infection in the secondary host may be either similar to that in the primary host, as in transmission from sheep to cattle, or severe, as in transmission from sheep to pregnant women.

Methods used for the diagnosis of chlamydial infections include demonstration of organisms in stained impression smears, immunohistochemistry, detection of chlamydial DNA by the polymerase chain reaction and isolation in cell culture or embryonated eggs. Animal infections can be confirmed by serology.

33 *Rickettsiales*



Organisms in the order *Rickettsiales* form a diverse group of non-motile, Gram-negative bacteria which replicate only in host cells. They can be cultured in the yolk sac of embryonated eggs or in selected tissue culture cell lines. In addition to host-cell dependence and poor affinity for basic dyes, a requirement for an invertebrate vector distinguishes them from most conventional bacteria.

At present, two families, *Rickettsiaceae* and *Anaplasmataceae*, comprise the *Rickettsiales*. Organisms in the family *Rickettsiaceae*, referred to as rickettsiae, generally target macrophages, leukocytes and endothelial cells. Species of veterinary importance in the family *Rickettsiaceae* are listed in Table 33.1. Members of the *Anaplasmataceae* parasitize erythrocytes and leukocytes and possess cytoplasmic membranes but lack cell walls (Table 33.2).

Animal hosts and arthropod vectors are the reservoirs of most rickettsiae. In arthropods, rickettsiae replicate in epithelial cells of the gut before spreading to the salivary glands and ovaries where further replication may occur. Organisms are transmitted when the arthropod feeds on the animal host. Some organisms are maintained in tick populations by transovarial transmission; trans-stadial but not transovarial transmission of *Ehrlichia canis* and *Anaplasma phagocytophilia* occurs in ticks. Transmission by flukes has been confirmed for *Neorickettsia* species. Apart from *Coxiella burnetii*, which produces endospore-like forms, most rickettsiae are labile outside host cells. Aerosol transmission of *C. burnetii* commonly occurs in domestic animals and humans.

Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*, a common rickettsial disease of humans, also affects dogs. These highly pathogenic organisms have a predilection for endothelial cells of small blood vessels. *Ehrlichia* species have a predilection for leukocytes. Members of the *Anaplasmataceae* have an affinity for erythrocytes and neutrophils. Tick-borne fever, caused by *Anaplasma phagocytophila*, affects domestic and wild ruminants in many European countries. Fever, inappetence and a reduced growth rate may be evident in young animals, while abortions or stillbirths may occur in naive, pregnant animals. Transient immunosuppression is a feature of the disease. *Coxiella burnetii* localizes and replicates in cells of the female reproductive tract and mammary glands of ruminants.

Members of the *Rickettsiales* can be recognized and differentiated by the species of animals affected, cell predilection, microscopic appearance and molecular techniques. Blood or tissue smears stained by the Giemsa technique can be used to demonstrate the morphology of many rickettsial organisms. They occur as purplish-blue, small individual organisms, sometimes in clusters. Fluorescent antibody techniques can be used to identify specific rickettsial organisms in smears. Some rickettsiae can be isolated in the yolk sac of embryonated eggs or in defined tissue culture cell

Table 33.1 Species of veterinary importance in the family *Rickettsiaceae*.

Pathogen	Hosts / Vectors	Disease	Geographical distribution
Coxiella burnetii	Humans, ruminants / aerosols, ticks	Q fever in humans, sporadic abortion in ruminants	Worldwide
Ehrlichia ruminantium	Ruminants / ticks	Heartwater	Sub-Saharan Africa, Caribbean islands
'*E. ovina*'	Sheep / ticks	Ovine ehrlichiosis	Africa, Asia, Middle East
E. canis	Dogs / ticks	Canine monocytic ehrlichiosis	Tropical and subtropical regions
E. ewingii	Dogs / ticks	Canine granulocytic ehrlichiosis	USA
'*E. ondiri*'	Cattle / ticks suspected	Bovine petechial fever	Highlands of East Africa
Neorickettsia risticii	Horses / flukes suspected	Potomac horse fever	North America, Europe
'*N. elokominica*'	Dogs, bears, racoons / flukes	Elokomin fluke fever	West coast of North America
N. helminthoeca	Dogs / flukes	Salmon poisoning disease	West coast of North America
Rickettsia rickettsii	Humans, dogs / ticks	Rocky Mountain spotted fever	North, Central and South America

lines. Molecular methods, including nucleic acid probes and polymerase chain reaction techniques, have been developed to detect some rickettsial pathogens.

Rickettsial organisms are relatively host-specific. Because definitive arthropod or fluke vectors are involved in the transmission of most rickettsiae, diseases associated with these organisms tend to occur in defined geographical regions. The clinical signs frequently reflect the targeting of a particular cell type by the causal rickettsial agent. Q fever and Rocky Mountain spotted fever are important zoonotic diseases. Q fever, caused by *Coxiella burnetii*, is an influenza-like occupational disease of those in contact with farm animals and their products. Most infections are acquired by inhalation of aerosols from parturient sheep, goats or cattle.

A limited number of vaccines are available for rickettsial pathogens. In many instances, arthropod vectors such as ticks are involved in pathogen transmission. For diseases transmitted in this manner, tick control is an essential part of disease prevention. Tetracycline therapy, administered early in the disease, may be effective. For some rickettsial diseases, such as Rocky Mountain spotted fever, treatment should be continued for up to two weeks.

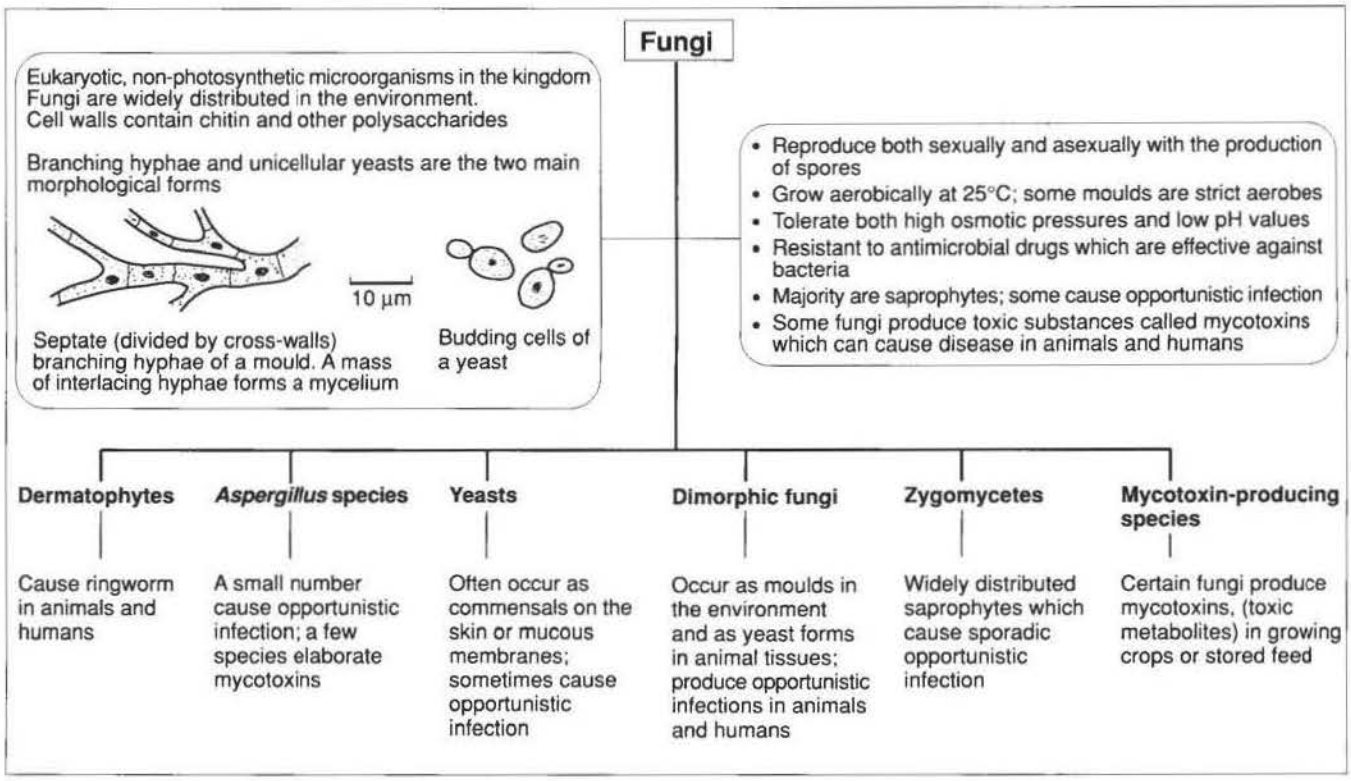
Table 33.2 Species of veterinary importance in the family *Anaplasmataceae*.

Pathogen	Hosts / Vectors	Disease	Geographical distribution
Aegyptianella pullorum	Poultry / ticks	Aegyptianellosis	Africa, Asia, Mediterranean region
Anaplasma marginale	Ruminants / ticks	Anaplasmosis	Tropical and subtropical regions
A. ovis	Sheep, goats / ticks	Anaplasmosis	Asia, Africa, Europe, USA
A. phagocytophila	Ruminants / ticks	Tick-borne fever	European countries
A. platys	Dogs / ticks suspected	Canine cyclic thrombocytopenia	USA, Israel
A. bovis	Cattle / ticks	Anaplasmosis	Africa, Middle East, Asia, South America
Eperythrozoon ovis	Sheep, goats / biting arthropods suspected	Eperythrozoonosis	Worldwide
Haemobartonella canis	Dogs / ticks suspected	Canine haemobartonellosis	Worldwide

Section III

Mycology

34 General features of fungi associated with disease in animals

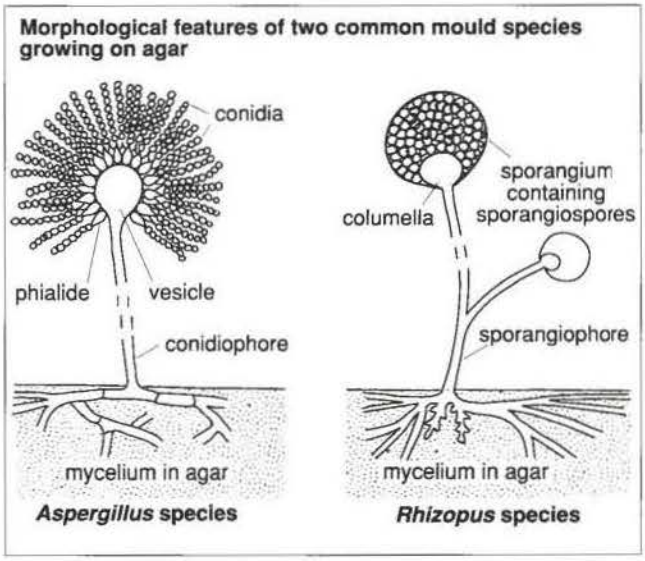


Fungi are eukaryotic, non-photosynthetic heterotrophs which produce exoenzymes and obtain nutrients by absorption. Although more than 250,000 species in the kingdom Fungi are recognized, less than 150 are known to be pathogenic for animals and humans. The two main morphological fungal forms are moulds and yeasts. Moulds grow as branching filaments called hyphae, whereas the unicellular yeasts have an oval or spherical appearance. Fungi grow aerobically. Incubation temperatures and time required for development of distinctive colonial morphology are indicated in Table 34.1. Dimorphic fungi occur in both mould and yeast forms. Environmental factors usually determine the form in which a dimorphic fungus occurs.

Fungal species may be saprophytic or parasitic. Saprophytic fungi, which are widespread in the environment and are involved in the decomposition of organic matter, occasionally

Table 34.1 Incubation conditions appropriate for fungal cultures.

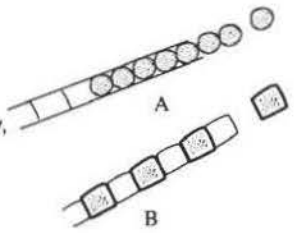
Fungal group	Incubation conditions	
	Temperature (°C)	Time
Dermatophytes	25	2 to 4 weeks
Aspergillus species	37	1 to 4 days
Yeasts (pathogenic)	37	1 to 4 days
Dimorphic fungi		
mould phase	25	1 to 4 weeks
yeast phase	37	1 to 4 weeks
Zygomycetes	37	1 to 4 days



Asexual spores produced by fungi of veterinary importance

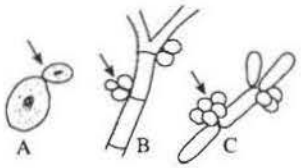
Arthroconidia (arthrospores)

Spores which are formed and subsequently released during the process of hyphal fragmentation. Spores may be formed successively, as in dermatophytes (A), or with intervening empty cells as in *Coccidioides immitis* (B)



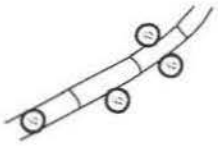
Blastoconidia (blastospores)

Conidia (arrows) which are produced by budding, as in *Candida albicans*, from a mother cell (A), from hyphae (B) or from pseudohyphae (C)



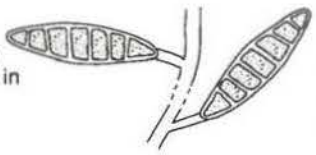
Chlamydoconidia (chlamydospores)

Thick-walled, resistant spores which contain storage products. These structures are formed by some fungi in unfavourable environmental conditions



Macroconidia

Large multi-celled conidia which are produced by dermatophytes in culture

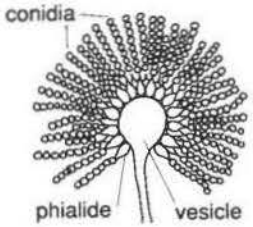


Microconidia

Small conidia which are produced by certain dermatophytes

Phialoconidia

Conidia produced from phialides. The phialides of *Aspergillus* species arise from a vesicle



Sporangiospores

Spores (arrow), formed by zygomycetes such as *Rhizopus* species, are released when a mature sporangium ruptures

cause sporadic, opportunistic infections in animals. The parasitic dermatophytes cause ringworm in animals. Overgrowth of yeasts, which are often commensals on skin and mucous membranes, sometimes causes localized lesions.

Hyphal cell walls are mainly composed of carbohydrate components including chitin macromolecules with cellulose cross-linkages. In yeasts, cell walls contain protein complexed with polysaccharides. Both moulds and yeasts have nuclei with well-defined nuclear membranes, mitochondria and networks of microtubules. Morphological features of two common mould species are illustrated.

Moulds tend to form large colonies with growth and extension of hyphae at their periphery. The two main types of spores produced by these fungi are conidia and sporangiospores. Conidia are formed on conidiophores and sporangiospores are formed within a sporangium, a sac-like structure. Asexual spores produced by fungi differ in appearance. In most yeasts, asexual division is by budding. Colonies of yeast-like fungi are soft, smooth and round. The pathogenic mechanisms whereby fungi produce disease are listed in Box 34.1. Factors which predispose to infection with fungi are indicated in Box 34.2.

Box 34.1 Mechanisms involved in fungal diseases

- Tissue invasion (mycosis)
- Toxin production (mycotoxicosis)
- Induction of hypersensitivity

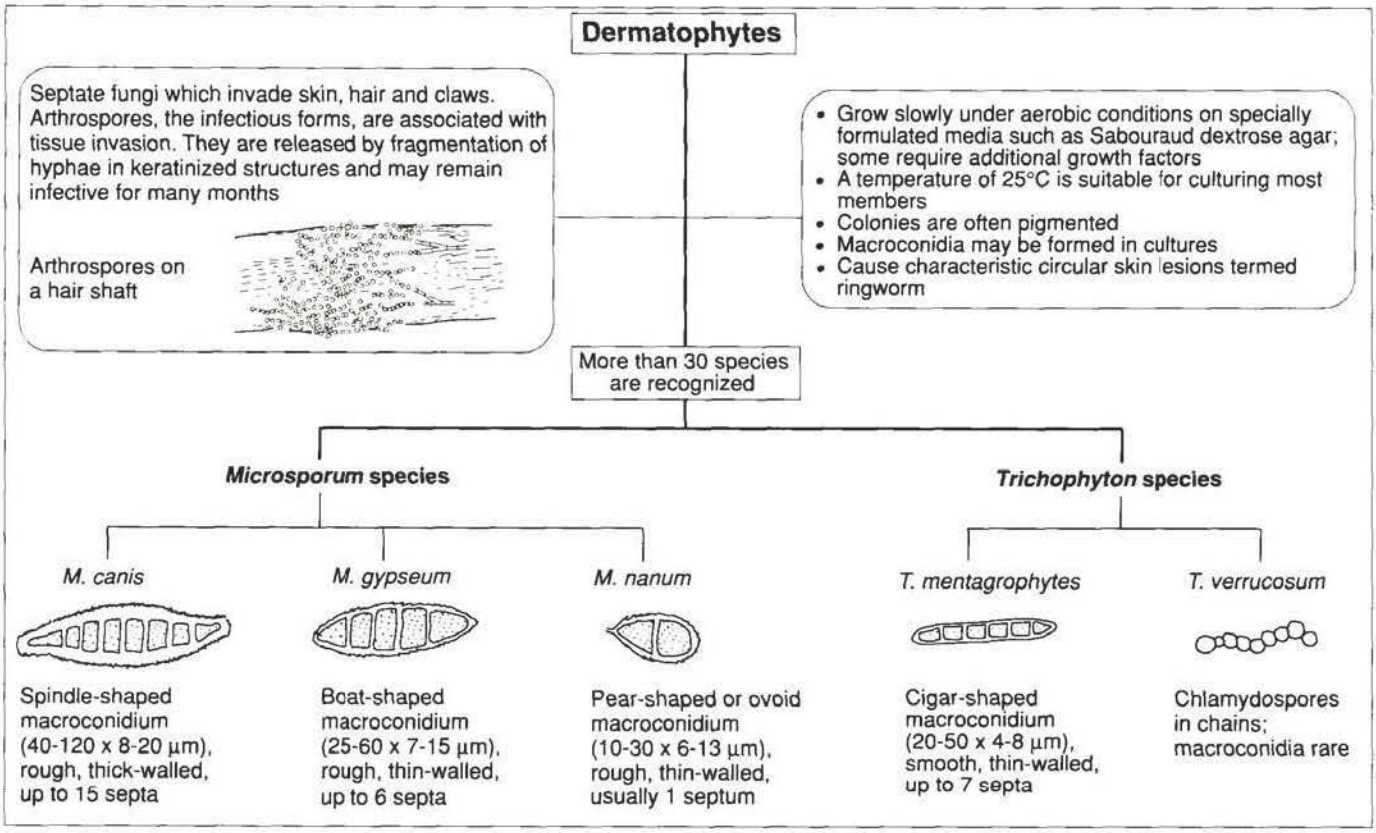
Box 34.2 Factors which may predispose to fungal invasion of tissues

- Immunosuppression
- Prolonged antibiotic therapy
- Immunological defects
- Immaturity, ageing and malnutrition
- Exposure to heavy challenge of fungal spores
- Traumatized tissues
- Persistent moisture on skin surface
- Some neoplastic conditions
- Immunosuppressive viral diseases such as panleukopenia and infectious peritonitis in cats

Methods of differentiating fungal species include examination of sporing heads for conidial arrangement or the presence of a sporangium. Features of vegetative hyphae used for differentiation include the presence or absence of septa and pigment. The size, appearance and colour of fungal colonies are useful for species differentiation. Yeasts can be differentiated by colonial appearance and by the size and shape of individual cells.

Options for antifungal chemotherapy include the polyenes nystatin and amphotericin B, and antifungal azoles such as fluconazole and ketoconazole. Griseofulvin, used for the treatment of ringworm, accumulates in keratinized tissues. Terbinafine is used for treating ringworm and sporotrichosis.

35 Dermatophytes



The dermatophytes are a group of septate fungi which occur worldwide and invade superficial keratinized structures such as skin, hair and claws. More than 30 species of dermatophytes are recognized. Most belong to the Fungi Imperfecti and are classified in three anamorphic genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. Arthrospores (arthroconidia), the infectious forms associated with tissue invasion by this group of fungi, are released by fragmentation of hyphae in keratinized structures. These resistant forms can remain viable for more than 12 months in suitable environments. Macroconidia and microconidia are produced in culture. The colonies of many dermatophytes are pigmented.

Dermatophytosis (ringworm) affects many animal species (Table 35.1). The disease is a zoonosis and many human infections are caused by *Microsporum canis*. Dermatophytes can be grouped on the basis of their habitats and host preferences as geophilic, zoophilic or anthropophilic. Zoophilic and anthropophilic dermatophytes are obligate pathogens of animals and humans respectively. Geophilic dermatophytes inhabit and replicate in soil. Animals can acquire infection with geophilic dermatophytes from soil or through contact with infected animals. Individual dermatophytes are identified mainly by colonial morphology and the microscopic appearance of macroconidia or other structures (Table 35.2). Dermatophytes invade keratinized structures such as the stratum corneum of the epidermis, hair follicles, hair shafts and feathers. Young, aged, debilitated and immunosuppressed animals are particularly susceptible to infection. Most infections in cats are caused by *Microsporum canis*. Clinical features of the disease include classical ringworm lesions, miliary dermatitis and, rarely, generalized lesions in immunosuppressed animals. Inapparent infections are known to occur in cats. In addition to infections caused by *M. canis*, dogs may become infected with *M. gypseum* and *Trichophyton mentagrophytes*. The disease usually presents as areas of alopecia, scaling and broken hairs surrounded by inflammatory zones. Generalized infections, which are uncommon in dogs, may be associated with conditions such as hyperadrenocorticism and immunosuppression. *Trichophyton verrucosum* is the usual cause of ringworm in cattle. Calves are most commonly affected and often develop characteristic lesions on the face and around the eyes. Oval areas of affected skin are alopecic with greyish-white crusts. Infection is most common in winter and a number of animals are usually involved. A vaccine composed of an attenuated strain of *T. verrucosum* has been used for the control of bovine dermatophytosis. *Trichophyton equinum* is the main cause of ringworm in horses. Transmission occurs by direct contact or from contaminated harness or grooming gear. Horses under four years of age are particularly susceptible to dermatophytosis.

Table 35.1 Dermatophytes of animals, their main hosts and reported geographical distribution.

Dermatophyte	Hosts	Geographical distribution
Microsporum canis var. *canis*	Cats, dogs	Worldwide
M. canis var. *distortum*	Dogs	New Zealand, Australia, North America
M. equinum	Horses	Africa, Australasia, Europe, North and South America
M. gallinae	Chickens, turkeys	Worldwide
M. gypseum	Horses, dogs, rodents	Worldwide
M. nanum	Pigs	North and South America, Europe, Australasia
Trichophyton equinum	Horses	Worldwide
T. equinum var. *autotrophicum*	Horses	Australia and New Zealand
T. mentagrophytes var. *mentagrophytes*	Rodents, dogs, horses and many other animal species	Worldwide
T. verrucosum	Cattle	Worldwide

Because diagnosis based on clinical signs is usually difficult, laboratory investigation of dermatophytosis is often necessary. As dermatophytes tend to parasitize particular hosts, the animal species affected may indicate the dermatophyte likely to be involved (Table 35.1). Specimens suitable for laboratory examination include plucked hair, deep skin scrapings from the edge of lesions, scrapings from affected claws and biopsy material. Hair and skin scrapings treated with 10% potassium hydroxide should be examined microscopically for the presence of arthrospores. Histological sections of skin can be stained by the PAS or methenamine silver techniques to demonstrate fungal structures. Specimens are cultured on Sabouraud dextrose agar, usually with the addition of yeast extract, chloramphenicol and cyclohexamide. Inoculated plates are incubated aerobically at 25°C and examined twice weekly for up to five weeks. Identification is based on colonial morphology and microscopic appearance of macroconidia. In cats and dogs with suspicious lesions, examination with a Wood's lamp should be carried out to detect infection with *M. canis*. A characteristic green fluorescence may be evident when infected hairs are exposed to UV light.

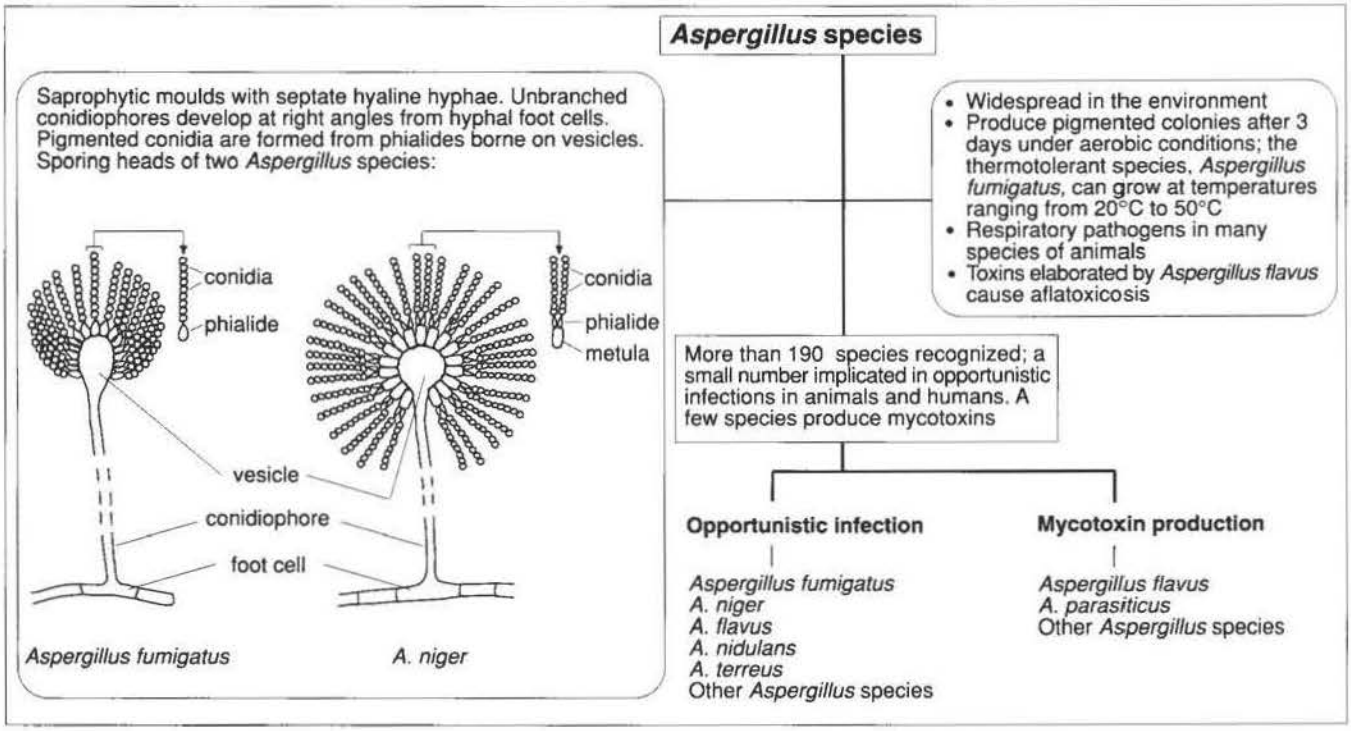
Treatment and control are particularly important in domestic carnivores because dermatophytoses are zoonoses. Animals with suspicious lesions should be isolated and early laboratory confirmation is essential. Removal of the hair coat by clipping may be necessary around affected areas, followed by topical treatment with miconazole shampoo. Clippings should be disposed of carefully. Oral treatment with itraconazole or other suitable antifungal drugs may be necessary if lesions are extensive. Contaminated bedding should be burnt and grooming equipment should be disinfected with 0.5% sodium hypochlorite.

Topical treatment with captan or natamycin may be effective in cattle. Affected horses should be treated topically and contaminated harness and grooming gear should be disinfected with 0.5% sodium hypochlorite.

Table 35.2 Colonial appearance and growth characteristics of dermatophytes isolated from animals.

Dermatophyte	Colonial appearance on Sabouraud dextrose agar	Comments
Microsporum canis	Obverse, white to buff with bright orange periphery; reverse, yellowish-orange or yellowish-brown	Heavy sporulation occurs on rice grain media. Colony size up to 50 mm after incubation for 10 days
M. gypseum	Obverse, buff to cinnamon with white border and powdery; reverse, buff to reddish-brown	Colony size up to 50 mm after incubation for 10 days. Mouse-like odour
M. nanum	Obverse, cream to tan and powdery; reverse, reddish brown	Colony size up to 35 mm after incubation for 10 days
Trichophyton equinum	Obverse, initially white and fluffy, later buff and folded; reverse, yellow to dark reddish-brown	Nicotinic acid required for growth. Colony size up to 35 mm after incubation for 10 days
T. mentagrophytes	Obverse, cream-tan to buff and powdery; reverse, buff-tan to dark brown	Colony size up to 30 mm after incubation for 10 days. Urease-positive; grows well at 37°C
T. verrucosum	Obverse, white, heaped and velvety; reverse, white or pale buff	Growth slow, colony size up to 10 mm after incubation for 20 days. Requires thiamine and sometimes inositol for growth. Grows at 37°C

36 *Aspergillus* species



Although the genus *Aspergillus* contains more than 190 species, only a limited number of these have been implicated in opportunistic infections in animals and humans. *Aspergillus* species are saprophytes which are widely distributed in the environment. *Aspergillus fumigatus* is the species most often involved in tissue invasion; other potentially invasive species include *A. niger*, *A. flavus* and *A. terreus*.

Aspergilli are aerobic and grow rapidly, forming distinct colonies after incubation for two to three days. The colour of the obverse side of colonies, which may be bluish-green, black, brown or yellow, varies with individual species and cultural conditions. *Aspergillus fumigatus*, a thermotolerant species, grows at temperatures ranging from 20°C to 50°C.

The sporing heads of two *Aspergillus* species are shown above. The hyphae are septate, hyaline and up to 8 µm in diameter. Unbranched conidiophores develop at right angles from specialized hyphal foot cells. The tip of the conidiophore enlarges to form a vesicle which becomes partially or completely covered with flask-shaped phialides. The phialides produce chains of round pigmented conidia which are up to 5 µm in diameter. Respiratory infection may occur following inhalation of spores. Occasionally, infection can result from ingestion of spores or following trauma. Systemic infection is invariably associated with immunosuppression. Species such as *A. flavus*, which elaborate potent toxins when growing on cereals and other crops, cause mycotoxicoses.

Aspergillus species grow on standard laboratory media such as Sabouraud dextrose agar. Because the genus contains a large number of species, differentiation is difficult. Colonies can be up to 5 cm in diameter after incubation for five days. The colonies of *A. fumigatus* become velvety or granular and bluish-green with narrow white peripheries. Colonies of *A. niger* are black and granular, features imparted by the large pigmented sporing heads. *Aspergillus flavus* colonies are yellowish-green with a fluffy texture.

Infection with *Aspergillus* species, mainly *A. fumigatus*, has been recorded in many species of animals. Aspergillosis, which is primarily a respiratory infection, follows spore inhalation. Immune competence of the host largely determines the outcome of infection. Factors which may modify immune competence include corticosteroid therapy and long-term treatment with antimicrobial drugs. Interference with both neutrophil and monocyte function may predispose to tissue invasion. Hyphal invasion of blood vessels leads to vasculitis and thrombus formation. Mycotic granulomas may develop in the lungs and occasionally in other internal organs.

Clinical cases of aspergillosis are comparatively uncommon and usually sporadic. The clinical conditions caused by *Aspergillus* species in domestic animals are summarized in Table 36.1. Brooder pneumonia affects newly-hatched

Table 36.1 Clinical conditions caused by *Aspergillus* species in domestic animals.

Hosts	Condition	Comments
Birds	Brooder pneumonia	Occurs in newly-hatched chickens in incubators
	Pneumonia and airsacculitis	Chickens and poults up to 6 weeks of age are most susceptible; older birds sometimes affected
	Generalized aspergillosis	Dissemination of infection usually from the respiratory tract
Horses	Guttural pouch mycosis	Confined to guttural pouch, often unilateral
	Nasal granuloma	Produces a nasal discharge and interferes with breathing. Fungi other than *Aspergillus* spp. may initiate this condition
	Keratitis	Localized infection following ocular trauma
	Intestinal aspergillosis	Enteric infection resulting in diarrhoea in foals
Cattle	Mycotic abortion	Occurs sporadically; produces thickened placenta and plaques on skin of aborted foetus
	Mycotic pneumonia	Uncommon condition of housed calves
	Mycotic mastitis	May result from the use of contaminated intramammary antibiotic tubes
	Intestinal aspergillosis	May cause acute or chronic diarrhoea in calves
Dogs	Nasal aspergillosis	Invasion of nasal mucosa and turbinate bones; occurs periodically
	Otitis externa	*Aspergillus* species may constitute part of a mixed infection
	Disseminated aspergillosis	Uncommon; may result in osteomyelitis or discospondylitis
Cats	Systemic aspergillosis	Rarely encountered; immunosuppressed animals are at risk

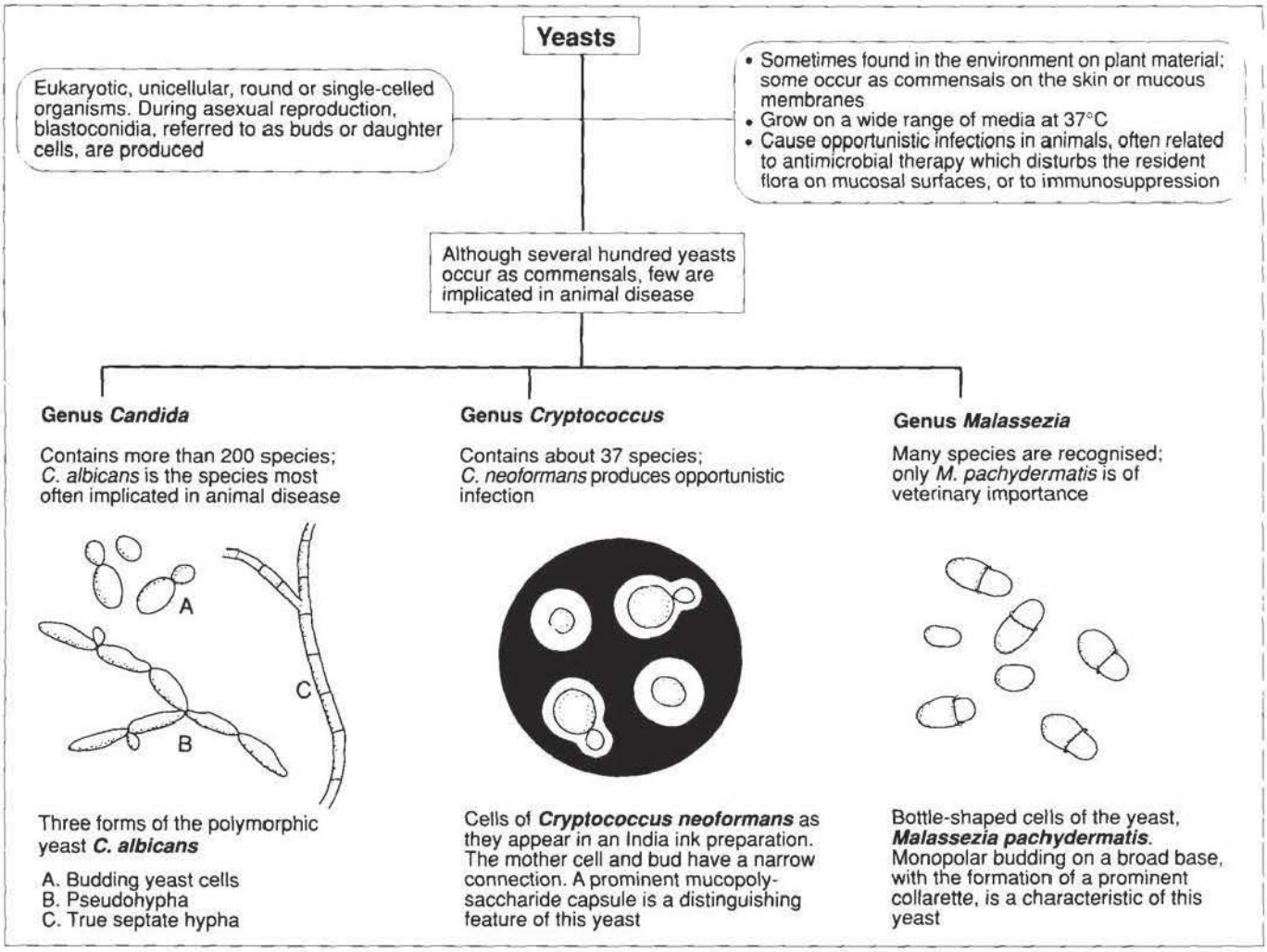
chickens which are exposed to high numbers of *A. fumigatus* spores. Affected chickens develop somnolence and inappetence and many may die. Yellowish nodules are present in the lungs, airsacs and, occasionally, in other organs. Histopathological evidence of tissue invasion by fungi and culture of *A. fumigatus* from lesions are required for confirmation. Strict hygiene and routine fumigation of incubators are effective control measures. Aspergillosis in mature birds frequently follows inhalation of spore-laden dust derived from contaminated litter or feed. Poultry and captive penguins, raptors and psittacine birds may be affected. Clinical signs, which are variable, include dyspnoea and emaciation. Yellowish nodules resembling lesions of avian tuberculosis can be observed in lungs and airsacs. Diagnosis is confirmed by histopathology and culture.

In horses, guttural pouch mycosis, which is frequently associated with *A. fumigatus* infection, is usually unilateral. Plaque-like lesions develop in the mucosa of the pouch wall. When fungal hyphae penetrate to deeper tissues they cause tissue necrosis, thrombosis, erosion of blood vessel walls and neural damage. Clinical signs include epistaxis, dysphagia and laryngeal hemiplegia. Diagnosis is based on clinical signs, radiographic evidence of fluid accumulation in the pouch and demonstration of characteristic lesions by endoscopy. Confirmation is based on the demonstration of fungal hyphae in biopsy specimens and isolation of *A. fumigatus* from lesions.

Nasal aspergillosis in dogs is encountered in young to middle-aged dolichocephalic breeds. Clinical signs, which are often unilateral, include persistent profuse sanguino-purulent nasal discharge with sneezing and bouts of epistaxis. Radiography may reveal an increased radiolucency of turbinate bones. Culture and histopathological examination of biopsy material are essential for confirmation.

Mycotic abortion in cows occurs sporadically and its prevalence may be influenced by poor quality contaminated fodder harvested in wet seasons. *Aspergillus fumigatus* can proliferate in damp hay, in poor quality silage and in brewer's grains. Infection, which reaches the uterus haematogenously, causes placentitis, leading to abortion late in gestation. Affected cows usually show no signs of systemic illness. Intercotyledonary areas of the placenta are thickened and leathery and the cotyledons are necrotic. Aborted foetuses may have raised cutaneous plaques, resembling ringworm lesions. Diagnosis is based on culture of *A. fumigatus* from foetal abomasal contents and histopathological evidence of mycotic placentitis.

37 Yeasts and disease production



Yeasts are eukaryotic, unicellular, round or oval, single-celled organisms. During asexual reproduction, blastoconidia, also referred to as buds or daughter cells, develop. Yeasts grow aerobically on Sabouraud dextrose agar and species capable of tissue invasion grow well at 37°C. Colonies, which are usually moist and creamy in texture, resemble large bacterial colonies. In addition to their environmental habitat on plants or plant material, yeasts also occur as commensals on the skin or mucous membranes of animals. Immunosuppression or factors such as antimicrobial therapy which disturb the resident flora on mucosal surfaces may facilitate yeast overgrowth leading to tissue invasion. Yeasts of importance in animal disease are *Candida* species (particularly *C. albicans*), *Cryptococcus neoformans* and *Malassezia pachydermatis*.

Candida species

Although there are more than 200 species in the genus *Candida*, the species most often implicated in animal disease is *Candida albicans*. It grows aerobically at 37°C on a wide range of media, including Sabouraud dextrose agar. Colonies are composed of budding oval cells approximately 5.0 x 8.0 µm. In animal tissues, *C. albicans* may exhibit polymorphism in the form of pseudohyphae or hyphae.

Candida albicans possesses a number of putative virulence factors. These include surface integrin-like molecules which allow adhesion to matrix proteins, surface structures which can bind fibrinogen and complement components, and enzymes such as proteases and phospholipases which may aid tissue invasion. Localized mucocutaneous tissue invasion can occur in the oral cavity or in the gastrointestinal and urogenital tracts. Predisposing factors include defects in cell-mediated immunity, concurrent disease, disturbance of the normal flora by prolonged use of antimicrobial drugs and damage to the mucosal surface from indwelling catheters. Opportunistic infections with *Candida* species, which occur sporadically, are usually associated with immunosuppression or the prolonged use of antimicrobial drugs. Overgrowth of commensal *Candida* species may result in localized mucosal damage in

parts of the digestive or urogenital tracts. The clinical conditions attributed to *Candida* species are presented in Table 37.1. Suitable specimens for culture and histopathology include biopsy or postmortem tissue samples and milk samples. Tissue sections, stained by the PAS or methenamine silver methods, may reveal budding yeast cells or hyphae. Culture is carried out aerobically at 37°C for up to five days on Sabouraud dextrose agar. Criteria used for identification of isolates include characteristic colonies yielding budding yeast cells, growth on media containing cyclohexamide, biochemical profile, germ tube production when incubated for two hours in serum at 37°C and chlamydospore production in cornmeal agar (specific for *C. albicans*).

Cryptococcus neoformans

Although the genus *Cryptococcus* contains about 37 species, only *C. neoformans* produces opportunistic infections. The yeast cells are round to oval and 3.5 to 8 µm in diameter. A daughter cell is formed as a bud from the mother cell on a narrow neck. When recovered directly from affected animals, the yeasts have thick mucopolysaccharide capsules which can be demonstrated in India ink preparations. *Cryptococcus* species are aerobic and form mucoid colonies on a variety of media, including Sabouraud dextrose agar. The ability to grow at 37°C distinguishes *C. neoformans* from other *Cryptococcus* species.

Infection with *C. neoformans* occurs through inhalation of yeast cells in contaminated dust. Some of these cells may be trapped in the nasal cavities or sinuses while others are deposited in the lungs. Virulence factors of *C. neoformans* include the capsule which is antiphagocytic, the ability to grow at mammalian body temperature and the production of phenol oxidase. Infection with *C. neoformans* is usually associated with defective cell-mediated immunity.

Table 37.1 Clinical conditions associated with *Candida albicans*.

Hosts	Clinical conditions
Pups, kittens, foals	Mycotic stomatitis
Pigs, foals, calves	Gastro-oesophageal ulcers
Calves	Rumenitis
Dogs	Enteritis, cutaneous lesions
Chickens	Thrush of the oesophagus or crop
Geese, turkeys	Cloacal and vent infections
Cows	Reduced fertility, abortion, mastitis
Mares	Pyometra
Cats	Urocystitis, pyothorax
Cats, horses	Ocular lesions
Dogs, cats, pigs, calves	Disseminated disease

Nasal, cutaneous, neural and ocular forms of cryptococcosis are recognized in cats. The disease in dogs, which is less common than in cats, is often disseminated with neurological and ocular signs. Surgical removal combined with parenteral antifungal drugs is the usual method for treating cutaneous cryptococcosis. Treatment with amphotericin B and flucytosine, ketoconazole, intraconazole or fluconazole may be of benefit. Therapy should continue for at least two months.

When first isolated, colonies of *Cryptococcus* species are mucoid due to the presence of capsular material. They may have a cream, tan or yellowish appearance. Budding yeasts with wide capsules can be demonstrated in India ink preparations. Most *Cryptococcus* species produce urease, rapidly hydrolyzing urea to ammonia. Identification criteria for *C. neoformans* include the ability to grow at 37°C, brown colonies on birdseed agar, and melanin demonstrable in cell walls using the Fontana-Masson stain on tissue sections.

Malassezia pachydermatis

Malassezia species, commensals on the skin of animals and humans, are aerobic, non-fermentative, urease-positive yeasts which grow at 35°C to 37°C. One species, *Malassezia pachydermatis*, is of veterinary importance. The cells of *M. pachydermatis*, which are bottle-shaped, thick-walled and up to 6.5 µm in length, reproduce by monopolar budding on a broad base. This yeast can be found on the skin of mammals and birds, particularly in areas rich in sebaceous glands.

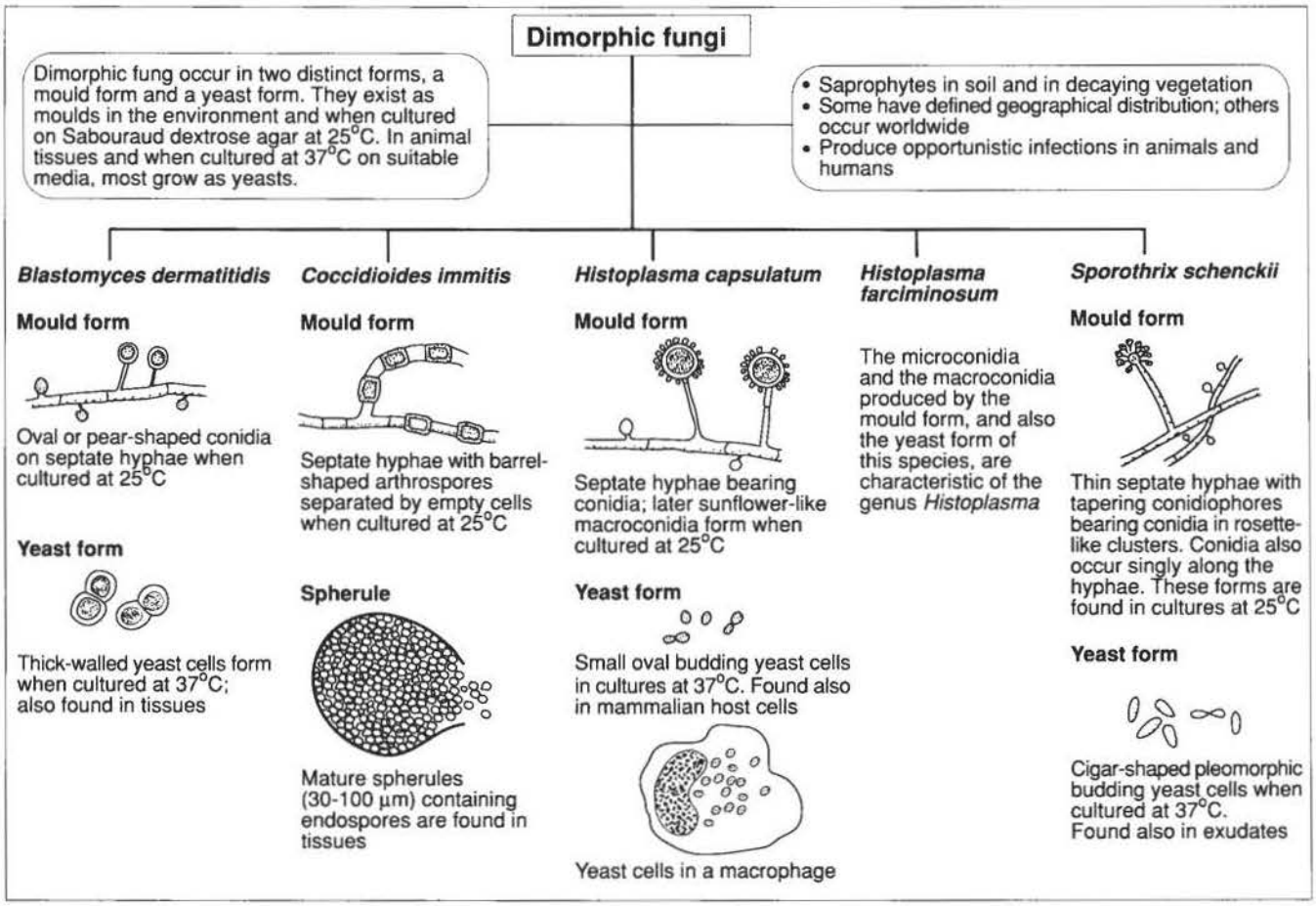
Malassezia pachydermatis is associated with two clinical conditions, otitis externa and dermatitis, usually in dogs. Colonization and growth of the organism in these locations may be associated with immunosuppression and other predisposing factors. When the yeast cells are present in high numbers, they apparently induce excessive sebaceous secretion, a feature of seborrhoeic dermatitis. In otitis externa, the production of proteolytic enzymes by *M. pachydermatis* results in damage to the epithelial lining of the ear canal.

Factors which predispose to canine seborrhoeic dermatitis include hypersensitivity disorders, keratinization defects, immunosuppression and persistently moist skin folds. Pruritis and erythema are accompanied by foul-smelling greasy exudate with matting of hair. Treatment with miconazole-chlorhexidine shampoo or a combination of topical and oral ketoconazole may be effective.

Canine otitis externa is characterized by a dark pungent discharge from the ear canal and intense pruritis, head shaking, scratching and rubbing of the ears. The epithelial lining of the ear canal is painful and swollen. Poor ear conformation, wax retention and immunosuppression are among the factors which may predispose dogs to the disease. *Malassezia pachydermatis*, which is present in low numbers in the ear canal of clinically normal dogs, may proliferate in otitis externa.

Characteristic yeast cells may be demonstrable in exudates stained with methylene blue. *Malassezia pachydermatis* can be cultured aerobically at 37°C for four days on Sabouraud dextrose agar containing chloramphenicol. Identification criteria include colonial appearance, growth without lipid supplementation and characteristic microscopic appearance.

38 Dimorphic fungi



Some fungi, referred to as dimorphic fungi, occur in two distinct forms, a mould form and a yeast form. They exist as moulds in the environment and when cultured on Sabouraud dextrose agar at 25°C to 30°C. In animal tissues, and when cultured at 37°C on brain-heart infusion agar with the addition of 5% blood, most grow as yeasts after conversion from the more stable mould form. The dimorphic fungi most often associated with disease in domestic animals are *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Coccidioides immitis*. The spores of these dimorphic fungi usually enter hosts by the respiratory route. A variant of *H. capsulatum*, referred to as *H. farciminosum*, generally enters through skin abrasions and produces lymphocutaneous lesions. *Sporothrix schenckii* also produces opportunistic infections in animals. Table 38.1 summarizes important features of dimorphic fungi associated with disease in animals and humans.

Blastomyces dermatitidis

Blastomycosis, caused by *B. dermatitidis*, most commonly affects dogs and humans. The disease is encountered in North America, Africa, the Middle East and India. Infection usually occurs by inhalation and pulmonary blastomycosis is the usual form of the disease. Presenting signs include coughing, exercise intolerance and dyspnoea. Amphotericin B, which may be combined with ketoconazole, is effective if administered early in the course of the disease.

When incubated at 25°C to 30°C on Sabouraud dextrose agar, mould colonies are white and cottony, usually becoming brown with age. When incubated at 37°C on brain-heart infusion agar with added cysteine and 5% blood, yeast colonies are cream to tan, wrinkled and waxy. Yeast cells may be demonstrated in cytological and histopathological preparations from affected tissues.

Histoplasma capsulatum

Although histoplasmosis, caused by *H. capsulatum*, occurs in many countries, it is endemic in the Mississippi and Ohio river valleys and in other areas of the USA. The dog and cat are the domestic species most often affected clinically. Epizootic lymphangitis, caused by *Histoplasma farciminosum*, occurs in *Equidae* in Africa, the Middle East and Asia.

Histoplasmosis in dogs and cats is probably associated with

Table 38.1 Dimorphic fungi which are associated with disease in animals and humans.

	Blastomyces dermatitidis	*Histoplasma capsulatum*	*Histoplasma farciminosum*	*Coccidioides immitis*	*Sporothrix schenckii*
Disease	Blastomycosis	Histoplasmosis	Epizootic lymphangitis	Coccidioidomycosis	Sporotrichosis
Geographical distribution	Eastern regions of North America, sporadic cases in India and the Middle East	Endemic in the Mississippi and Ohio river valleys, sporadic cases in some countries	Africa, Middle East, Asia	Semi-arid regions of southwestern USA, Mexico, Central and South America	Worldwide, most common in subtropical and tropical regions
Usual habitat	Acid soil rich in organic matter	Soil enriched with bat or bird faeces	Soil	Desert soils at low elevation	Dead vegetation, rose thorns, wooden posts, sphagnum moss
Main hosts	Dogs, humans	Dogs, cats, humans	Horses, other *Equidae*	Dogs, horses, humans	Horses, cats, dogs, humans
Site of lesions	Lungs, metastases to skin and other tissues	Lungs, metastases to other organs	Skin, lymphatic vessels, lymph nodes	Lungs, metastases to bones	Skin, lymphatic vessels

impaired cell-mediated immunity. Granulomatous lesions may be found in the lungs of both dogs and cats. Clinical signs in affected dogs include a chronic cough, persistent diarrhoea and emaciation. Ketoconazole and amphotericin B can be used for treatment.

Epizootic lymphangitis, caused by *H. farciminosum*, is a contagious disease. Horses usually acquire infection from environmental sources through minor skin abrasions on the limbs. Characteristic lymphocutaneous lesions consist of ulcerated discharging nodules, usually located along the course of thickened, hard, lymphatic vessels. Yeast cells of *H. farciminosum* are found in large numbers in lesions, mainly in macrophages. When cultured at 25°C to 30°C on Sabouraud dextrose agar, the mould form of *H. capsulatum* grows as white to buff colonies with aerial hyphae. Septate hyphae bear small conidia and in mature colonies sunflower-like macroconidia may be present. When cultured at 37°C on brain-heart infusion agar with added cysteine and 5% blood, yeast colonies are round, mucoid and cream-coloured. Budding yeast cells are oval to spherical. Histopathological examination of affected tissues reveals pyogranulomatous foci containing yeast forms.

Coccidioides immitis

The geophilic fungus, *C. immitis*, can infect many animal species including humans. Although grouped with the dimorphic fungi, *C. immitis* is biphasic rather than dimorphic because typical yeast forms are not produced. Large spherules containing endospores develop in tissues. Respiratory infections may follow inhalation of arthrospores.

Clinical infections caused by *C. immitis* are limited to defined arid regions of southwestern USA, Mexico, Central and South America. The domestic species most often affected is the dog. Canine coccidioidomycosis may present with non-specific signs including coughing, fever and inappetence. Dissemination from pulmonary lesions often results in osteomyelitis and lameness. Ketoconazole therapy for at least six months may be effective.

When cultured on Sabouraud dextrose agar at 25°C to 30°C, colonies are shiny, moist and grey, becoming white and cottony. Thick-walled, barrel-shaped arthroconidia, separated by empty cells are formed. Spherules of *C. immitis* may be demonstrated in exudates or aspirates cleared with 10% KOH. These structures may also be identified in stained tissue sections.

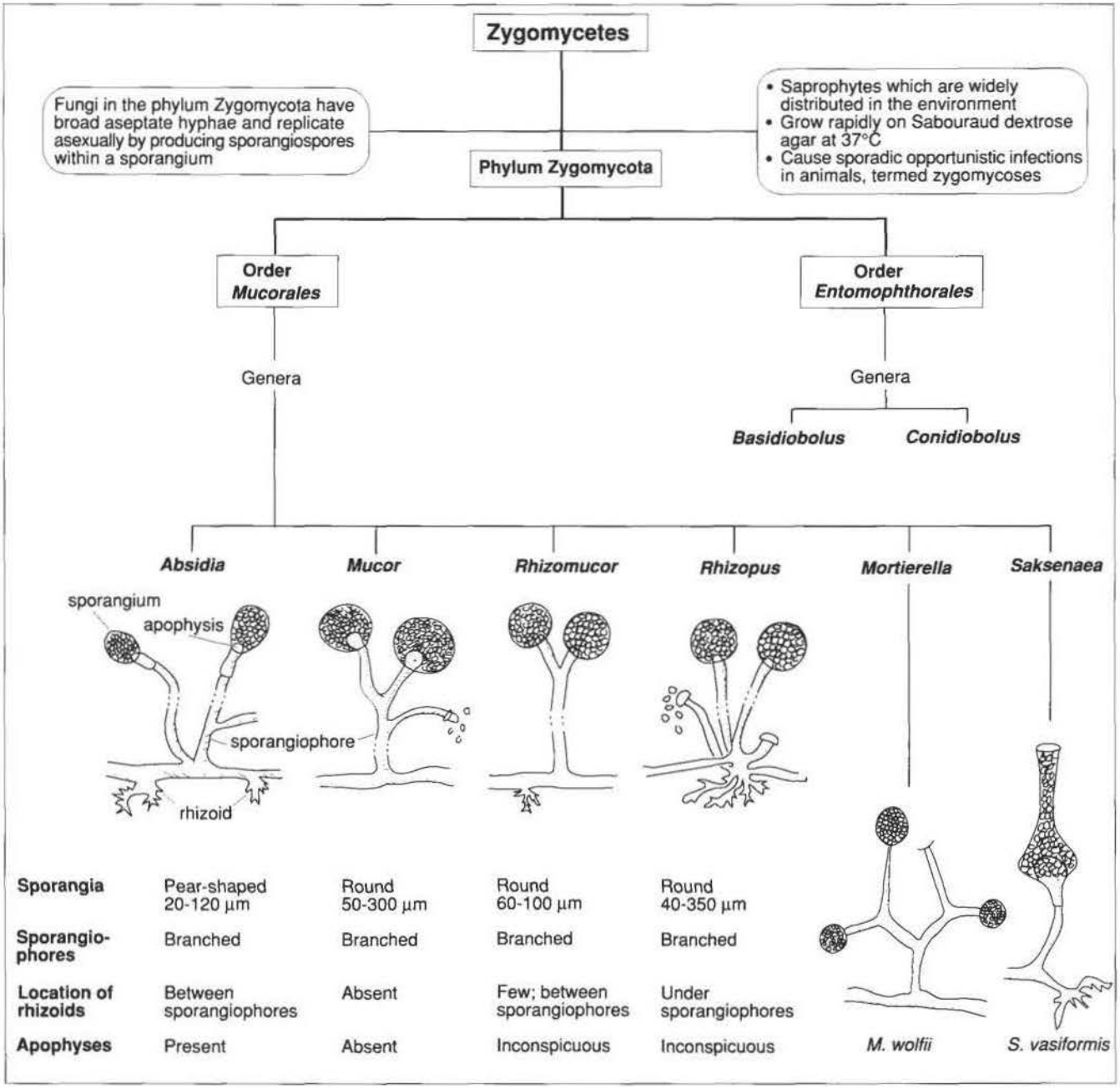
Sporothrix schenckii

This saprophytic fungus, which is widely distributed in the environment, grows on dead or senescent vegetation. Infections caused by *S. schenckii* occur sporadically in horses, cats, dogs and humans.

Sporotrichosis is a chronic cutaneous or lymphocutaneous disease which rarely becomes generalized. Lymphocutaneous sporotrichosis is the most common form of the disease in horses. Fungal spores usually enter through abrasions in the lower limbs, and nodules, which ulcerate and discharge a yellowish exudate, develop along the course of superficial lymphatic vessels. Subcutaneous oedema in the affected limb may result from lymphatic obstruction. In feline sporotrichosis, nodular skin lesions occur most often on limb extremities, head and tail. Nodules ulcerate and discharge a seropurulent exudate. Sporotrichosis in dogs often manifests as multiple, ulcerated and crusted alopecic cutaneous lesions over the head and trunk. Sporotrichosis may be treated with sodium iodide.

When cultured on Sabouraud dextrose agar at 25°C, mould colonies grow rapidly and are white, becoming black or brown, wrinkled and leathery. When cultured at 37°C on brain-heart infusion agar containing 5% blood, cream or tan colonies develop within three weeks. Direct microscopic examination of exudates from feline lesions usually reveals large numbers of cigar-shaped yeast cells. In exudates from other animals, yeast cells are sparse.

39 Zygomycetes of veterinary importance



Fungi in the phylum Zygomycota usually have broad (up to 15 µm in diameter) aseptate hyphae and replicate asexually by producing sporangiospores within a sporangium.

Two orders in the phylum, *Mucorales* and *Entomophthorales*, are of veterinary significance. Genera in these orders contain potentially pathogenic species. These rapidly-growing fungi, which are widely distributed saprophytes, can cause sporadic opportunistic infections in animals.

Infection with these fungi is uncommon in healthy immunocompetent animals. Factors which may predispose to infection include immunodeficiency, corticosteroid therapy, prolonged administration of broad spectrum antibiotics and infection with immunosuppressive viral pathogens (Box 39.1). Following ingestion or inhalation of spores from a contaminated environ-

ment, hyphae invade the mucosa, submucosa and local vessel walls, producing an acute necrotizing thrombotic vasculitis. Chronic lesions are usually localized and granulomatous.

Clinical infections

The zygomycoses of domestic animals are presented in Table 39.1. Apart from *Mortierella wolfii*, which may produce abortion followed by acute pneumonia, members of the *Mucorales* rarely cause recognizable disease syndromes in animals. Mycotic lesions caused by members of the *Mucorales* are less commonly encountered than those caused by *Aspergillus* species. Laboratory procedures, including isolation of the fungus and demonstration of hyphae in affected tissues, are essential for the diagnosis of zygomycoses.

Although *Aspergillus* species account for the majority of abortion cases in cattle, *M. wolfii*, *Absidia* species, *Mucor* species and *Rhizopus* species have been implicated. Mycotic abortion, which usually occurs late in gestation, is often linked to the feeding of mouldy hay or silage. The location of lesions on cotyledons suggests haematogenous infection of the uterus. The cotyledons are enlarged and necrotic and the intercotyledonary placental tissue is thickened and leathery. Occasionally, lesions may be observed grossly on the skin of aborted foetuses.

Mycotic rumenitis in cattle may follow mucosal damage associated with ruminal lactic acidosis. The microscopic appearance of the causal fungi in ruminal lesions suggests that in most cases, zygomycetes are involved. Infarction due to thrombotic arteritis, necrosis and haemorrhage are major features of the mycotic lesions. Zygomycotic abomasitis in calves, which may follow neonatal infection, can also produce perforation and peritonitis.

Two genera in the *Entomophthorales*, *Basidiobolus* and *Conidiobolus* are sometimes associated with opportunistic infections in animals. *Basidiobolus* species and *Conidiobolus* species are saprophytes in soil and in decaying vegetation. The route of entry of these fungi is probably through minor abrasions in the skin or nasal mucous membranes. Granulomatous lesions result from infection with these opportunistic pathogens. *Basidiobolus* species cause cutaneous lesions in the horse, while *Conidiobolus* species cause nasal granulomas in horses, sheep and llamas.

Specimens for laboratory examination should include biopsy or postmortem tissues for histopathology and culture. Staining of tissue sections by the PAS or methenamine silver techniques facilitates detection of hyphae. Isolation is carried out on Sabouraud dextrose agar without cyclohexamide. Cultures are incubated aerobically at 37°C for up to five days. Growth of *Absidia*, *Mucor*, *Rhizomucor* and *Rhizopus* species is rapid, filling the Petri dish with greyish or brownish-grey fluffy colonies within a few days. *Mortierella wolfii* has characteristic white velvety colonies with lobulated outlines after incubation for four days.

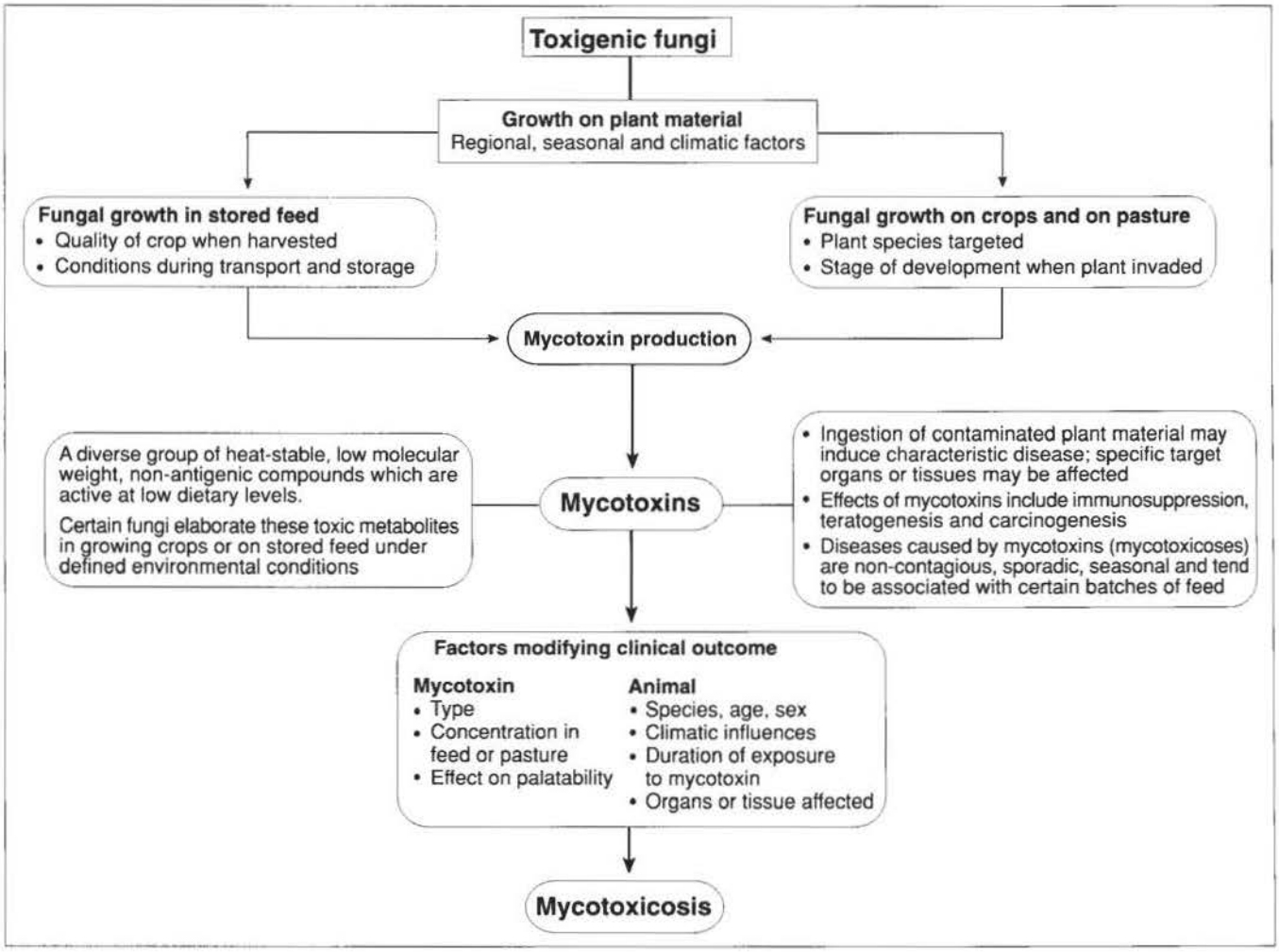
Table 39.1 Zygomycoses of domestic animals.

Fungal disease	Hosts	Clinical conditions
Mucormycosis	Cattle	Mesenteric and mediastinal lymphadenitis Abortion Pneumonia, following abortion caused by *Mortierella wolfii* Oesophagitis and enteritis in calves Rumenitis, abomasal ulcers Cranial granuloma
	Pigs	Enteritis in piglets Mesenteric and mandibular lymphadenitis Gastrointestinal ulcers
	Cats	Focal necrotizing pneumonia Necrotic enteritis
	Dogs	Enteritis
Entomophthomycosis	Horses	Cutaneous granulomas caused by *Basidiobolus* species Nasal granulomas caused by *Conidiobolus* species
	Dogs	Gastrointestinal and pulmonary granulomas caused by *Basidiobolus* species Subcutaneous granulomas caused by *Conidiobolus* species
	Sheep	Nasal granulomas caused by *Conidiobolus* species

Box 39.1 Factors which may predispose to zygomycoses

- Immunodeficiency
- Corticosteroid therapy
- Prolonged administration of broad-spectrum antibiotics
- Immunosuppressive viral diseases such as panleukopenia and infectious peritonitis in cats

40 Mycotoxins and mycotoxicoses 1



Mycotoxins, secondary metabolites of certain fungal species, are produced when toxigenic strains of fungi grow under defined conditions on crops, pasture or stored feed. The acute or chronic intoxication following ingestion of contaminated plant material is termed mycotoxicosis. More than 100 fungal species, many of them belonging to the genera *Penicillium*, *Aspergillus* and *Fusarium*, are known to elaborate mycotoxins. For fungal growth and toxin production, a suitable substrate must be available, along with moisture and optimal temperature and oxygen levels.

Mycotoxins are non-antigenic, low molecular weight compounds. Many are heat-stable, retaining toxicity following exposure to the processing temperatures used for pelleting and other procedures (Box 40.1). A particular mycotoxin may be produced by a number of fungal species and some fungi can elaborate several mycotoxins which may differ in biological activities, producing complex clinical effects. The severity of clinical signs is influenced by the period of exposure to contaminated feed and the amount of mycotoxin ingested. Some mycotoxins produce clinical signs relating to alterations in functioning of the central nervous system. Immuno-

Box 40.1 Characteristics of mycotoxins

- Low molecular weight, heat-stable substances
- Unlike many bacterial toxins, non-antigenic; exposure does not induce a protective immune response
- Many are active at low dietary levels
- Specific target organs or tissues affected
- Toxic effects include immunosuppression, mutagenesis, teratogenesis and carcinogenesis
- Accumulation in tissues of food-producing animals or excretion in milk may result in human exposure

Box 40.2 Epidemiological and clinical features of mycotoxicoses

- Outbreaks usually seasonal and sporadic
- No evidence of lateral spread to in-contact animals
- Certain types of pasture or stored feed may be involved
- Clinical presentation is usually ill-defined
- Severity of clinical signs is influenced by the amount of mycotoxin ingested; recovery is related to duration of exposure
- Antimicrobial medication is ineffective
- Confirmation requires demonstration of significant levels of mycotoxin in feed or in tissues from affected animals

suppression, mutagenesis, neoplasia or teratogenesis may also result from exposure. Epidemiological and clinical features of mycotoxicoses are summarized in Box 40.2.

Aflatoxicosis

Ingestion of aflatoxins, a large group of difuranocoumarins produced by toxigenic strains of *Aspergillus flavus*, *A. parasiticus* and some other *Aspergillus* species, can cause aflatoxicosis. Aflatoxins B_1, B_2, G_1 and G_2, are particularly important in disease production. After absorption from the gastrointestinal tract, aflatoxins are metabolized in the liver to a range of toxic and non-toxic products. Toxicity relates to binding of metabolites to macromolecules, especially nucleic acid and nucleoproteins. Toxic effects include reduced protein synthesis, carcinogenesis, teratogenesis and depressed cell-mediated immunity.

Clinical signs of disease are usually vague. Epidemiological features and postmortem findings may be of diagnostic value. Aflatoxin may be demonstrated in tissues obtained postmortem. Procedures for aflatoxin detection include thin-layer chromatography, high performance liquid chromatography, immunoassay techniques and biological assays.

Ergotism

Ingestion of toxic levels of certain ergopeptide alkaloids found in the sclerotia of *Claviceps purpurea* can cause ergotism. This disease occurs worldwide in many domestic animal species and in humans. The fungus colonizes the seed-heads of ryegrasses, rye and other cereals. The most important ergopeptide alkaloids in the sclerotia are ergotamine and ergometrine. These alkaloids have a number of pharmacological effects including direct stimulation of adrenergic nerves supplying arteriolar smooth muscle.

Convulsive ergotism, an acute form of the disease, is occasionally observed in ruminants. Small amounts of mycotoxins absorbed over relatively long periods result in persistent arteriolar constriction and endothelial damage. Swelling and redness of body extremities accompanied by lameness is followed by terminal gangrene. Ergotism can often be diagnosed clinically and the presence of ergots in pasture grasses or grain provides supporting evidence. The presence of alkaloids can be confirmed by chromatography.

Facial eczema

This economically important disease of sheep and cattle occurs in Australia, New Zealand and South Africa. The skin lesions develop as a result of photosensitization following exposure to the hepatotoxin sporidesmin in the spores of the saprophytic fungus *Pithomyces chartarum*.

During warm, humid conditions in late summer or early autumn, the fungus sporulates prolifically on pasture litter. Hepatobiliary lesions develop as a result of the accumulation and concentration of sporidesmin in the bile. Necrosis of biliary epithelium results in obstruction of intrahepatic ducts. The reduced capacity of the liver to excrete phylloerythrin, a potent photodynamic compound formed from chlorophyll by enteric organisms, results in photosensitization. In sheep, lesions develop in non-pigmented areas not covered by wool. Jaundice is usually present. In cattle, lesions are limited to areas of non-pigmented skin. Milk production may be seriously affected. In ruminants, photosensitization accompanied by jaundice is suggestive of the disease. Elevated liver enzymes are found in affected animals. Using competitive ELISA techniques, sporidesmin can be detected in many body fluids.

Mycotoxic oestrogenism

Zearalenone is a potent non-steroidal oestrogen produced by certain *Fusarium* species, particularly *F. graminearum*, when growing on stored maize and other cereals. Pasture levels of zearalenone in some countries may be sufficient to cause reproductive problems in cattle and sheep. Pigs, particularly prepubertal gilts, may be affected within weeks of ingesting contaminated food. Vulval oedema, hypertrophy of the mammary glands and uterus are features of the disease in gilts. In multiparous sows, anoestrus, pseudopregnancy and infertility may suggest oestrogenism. The mycotoxin can be detected by chromatography. Oestrogen activity in feed can be assayed by injection of extracts into sexually immature mice. An ELISA technique has been developed for detecting zearalenone in pasture samples and urine.

Tremorgen intoxications

Tremorgens, a heterogeneous group of mycotoxins, produce neurological effects including muscular tremors, ataxia, incoordination and convulsive seizures following ingestion.

Perennial ryegrass staggers is one of the most common mycotoxicoses of ruminants in horses in New Zealand, Australia, Europe and the USA. *Acremonium lolii*, growing on perennial ryegrass, produces lolitrems which are responsible for the clinical signs. Although morbidity may be high in affected herds or flocks, deaths are rare and recovery is rapid if animals are moved from contaminated pasture.

Paspalum staggers is caused by the ingestion of tremorgens present in the sclerotia of *Claviceps paspali* which are found in the seed-heads of paspalum grasses. The mycotoxins, paspalinine and paspalitrems A and B produce typical tremorgen ataxia. Many *Penicillium* species and some *Aspergillus* species produce tremorgens when growing on pasture plants or stored feed. The clinical signs resemble those which occur with ryegrass staggers.

41 Mycotoxins and mycotoxicoses 2

Mycotoxicoses of defined veterinary importance are presented in Table 41.1. The severity of clinical signs is influenced by the duration of exposure to contaminated feed or pasture, the effect of the mycotoxin on palatability, the amount of mycotoxin ingested and the organs or tissues affected. The role of a number of mycotoxins in disease production is not yet clearly defined and the pathogenesis of many diseases with suspected links to mycotoxins is poorly understood. It is probable that a number of diseases currently defined by their clinical presentation will be grouped with the mycotoxicoses in the future.

Table 41.1 Mycotoxicoses of domestic animals.

Disease / Mycotoxins	Fungus / Crop or substrate	Species affected / Geographical distribution	Functional or structural effects / Clinical findings
Aflatoxicosis / Aflatoxins B_1, B_2, G_1, G_2	*Aspergillus flavus*, *A. parasiticus* / Maize, stored grain groundnuts, soyabeans	Pigs, poultry, cattle, dogs, trout / Worldwide	Hepatotoxicity, immunosuppression, mutagenesis, teratogenesis, carcinogenesis / Ill-thrift, drop in milk yield, rarely death from acute toxicity
Diplodiosis / Unidentified neurotoxin	*Diplodia maydis* / Maize cobs	Sheep, cattle, goats, horses / South Africa	Neurotoxicity / Ataxia, paresis and paralysis in adults, perinatal deaths in lambs and calves
Ergotism / Ergotamine, ergometrine, ergocristine	*Claviceps purpurea* / Seed-heads of ryegrass and other grasses, cereals	Cattle, sheep, deer, horses, pigs, poultry / Worldwide	Neurotoxicity and vasoconstriction / Convulsions, gangrene of extremities, agalactia, hyperthermia in hot climates
Facial eczema / Sporidesmin	*Pithomyces chartarum* / Pasture litter from ryegrass and white clover	Cattle, sheep, goats / New Zealand, Australia, South Africa, South America, occasionally USA and parts of Europe	Hepatotoxicity, biliary occlusion / Photosensitization, jaundice
Fescue toxicosis / Ergovaline	*Neophytodium coenophialum* / Tall fescue grass	Cattle, sheep, horses, / New Zealand, Australia, USA, Italy	Vasoconstriction / Dry gangrene in cold weather in cattle and sheep (fescue foot); hyperthermia and low milk yields (fescue summer toxicosis)
Leukoencephalomalacia / Fumonisins B_1, B_2, A_1, A_2	*Fusarium moniliforme*, other *Fusarium* species / Standing or stored maize	Horses, other *Equidae*, pigs / Egypt, South Africa, USA, Greece	Liquefactive necrosis in cerebrum / Neurological signs of varying severity
Mouldy sweet potato toxicosis / Derivative of 4-ipomeanol	*Fusarium solani*, *F. oxysporum* / Sweet potatoes	Cattle / USA, Australia, New Zealand	Cytotoxicity producing interstitial pneumonia and pulmonary oedema / Respiratory distress, sudden death may occur
Mycotoxic lupinosis / Phomopsins A, B	*Phomopsis leptostromiformis* / Growing lupins with stem blight	Sheep, occasionally cattle, horses, pigs / Worldwide	Hepatotoxicity / Inappetence, stupor, jaundice, ruminal stasis, often fatal
Ochratoxicosis / Ochratoxins A, B, C	*Aspergillus ochraceus*, other *Aspergillus* species, *Penicillium viridicatum*, other *Penicillium* species / Stored barley, maize and wheat	Pigs, poultry / Worldwide	Degenerative renal changes / Polydipsia and polyuria in pigs, fall in egg production in birds
Oestrogenism / Zearalenone	*Fusarium graminearum*, other *Fusarium* species / Stored maize and barley, pelleted cereal feeds, maize silage	Pigs, cattle, occasionally sheep / Worldwide	Oestrogenic activity / Hyperaemia and oedema of vulva and precocious mammary development in young gilts; anoestrus and reduced litter size in mature sows; reduced fertility in cattle and sheep

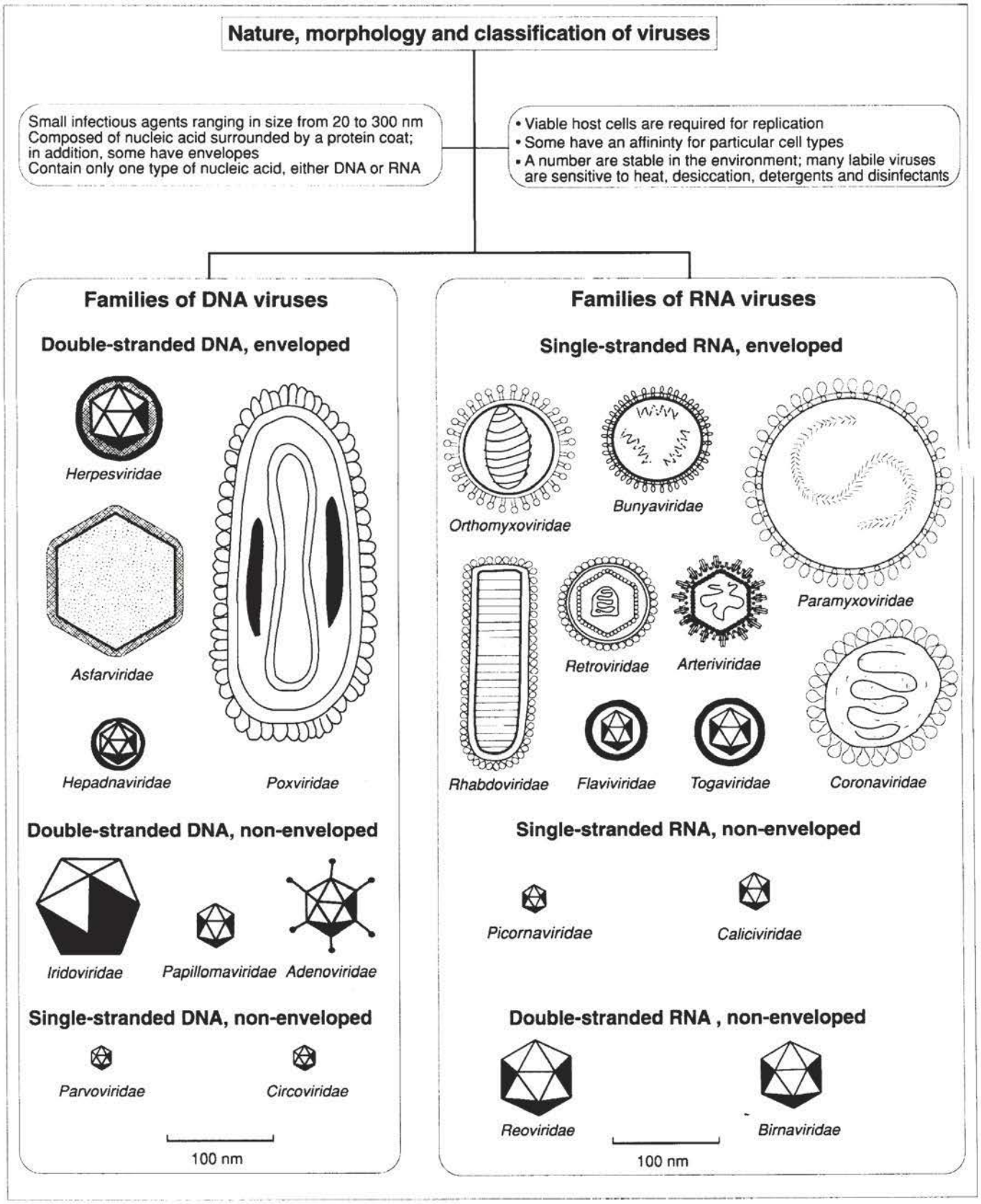
Table 41.1 Mycotoxicoses of domestic animals. (*continued*)

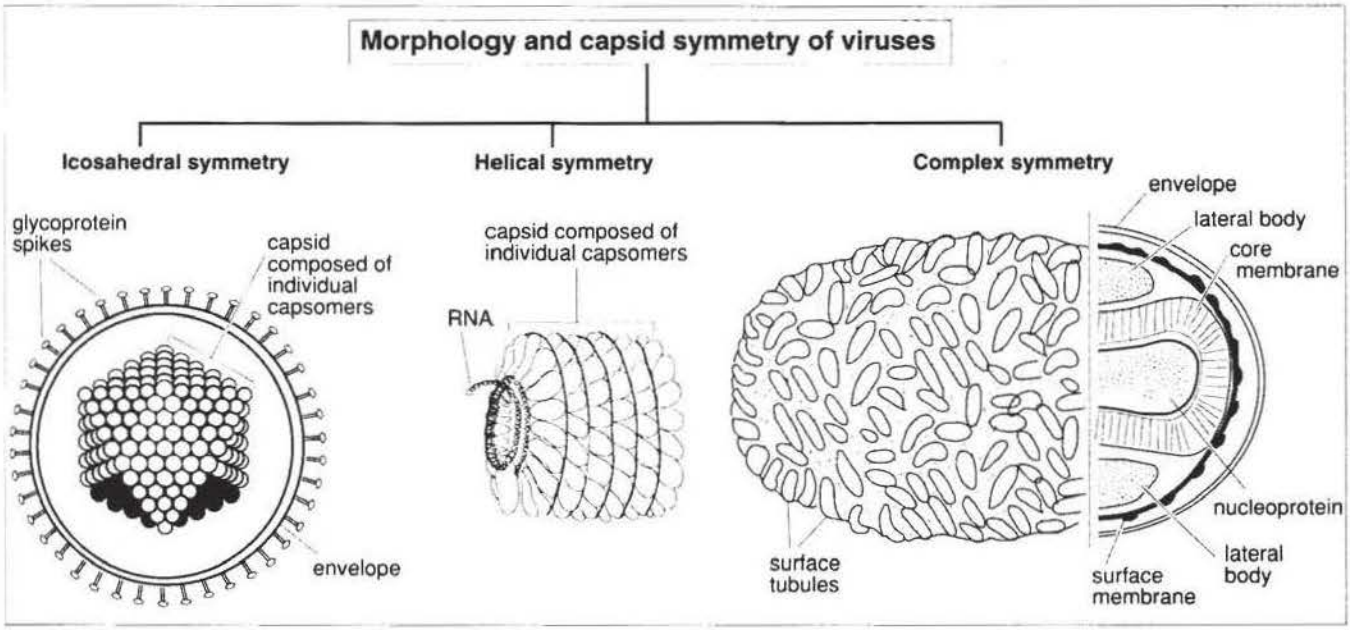
Disease / Mycotoxins	Fungus / Crop or substrate	Species affected / Geographical distribution	Functional or structural effects / Clinical findings
Porcine pulmonary oedema / Fumonisins B_1, B_2	*Fusarium* species / Maize	Pigs / USA, South Africa	Pulmonary oedema, hydrothorax / Cyanosis, death
Slaframine toxicosis / Slaframine	*Rhizoctonia leguminicola* / Legumes, especially red clover, in pasture or hay	Sheep, cattle, horses / USA	Cholinergic activity / Salivation, lacrimation, bloating, diarrhoea, sometimes death
Tremorgen intoxications			
Perennial ryegrass staggers / Lolitrem B	*Acremonium lolii* / Perennial ryegrass	Cattle, pigs, poultry, sheep, horses, deer / USA, Australia, New Zealand, Europe	Neurotoxicity / Muscular tremors, incoordination, convulsive seizures, collapse
Paspalum staggers / Paspalinine, paspalitrems A, B	*Claviceps paspali* / Seed-heads of paspalum grasses	Cattle, sheep, horses / New Zealand, Australia, USA, South America	Neurotoxicity / Muscular tremors, incoordination, convulsive seizures, collapse
Penitrem staggers / Verruculogen, paxilline, other mycotoxins	Many *Penicillium* species, some *Aspergillus* species / Stored feed and pasture	Ruminants, other domestic animals / Probably worldwide	Neurotoxicity / Muscular tremors, incoordination, convulsive seizures, collapse
Aspergillus clavatus-induced tremors / Unidentified neurotoxin	*Aspergillus clavatus* / Sprouted wheat, miller's malt culms	Cattle / China, South Africa, Europe	Neurotoxicity, degeneration of neurons / Frothing from mouth and knuckling of limbs when forced to move
Trichothecene toxicoses			
Food refusal and emetic syndrome / Vomitoxin (deoxynivalenol)	*Fusarium graminearum*, other *Fusarium* species / Cereal crops	Pigs, rarely other species / Countries with temperate or cold climates	Neurotoxicity / Contaminated feed refused, vomition, poor growth
Haemorrhagic syndrome / T-2 toxin, diacetoxy-scirpenol	*Fusarium graminearum*, *F. sporotrichoides*, other *Fusarium* species / Cereals, straw	Cattle, pigs, poultry / USA	Coagulopathy, immunosuppression / Necrotic skin lesions, necrotic lesions in alimentary tract, haemorrhages
Stachybotryotoxicosis / Satratoxin, roridin, verrucarin	*Stachybotrys atra* / Stored cereals, straw, hay	Horses, cattle, sheep, pigs / Former USSR, Europe, South Africa	Cytotoxicity, coagulopathy, immunosuppression / Stomatitis, necrotic lesions in alimentary tract, haemorrhages
Myrotheciotoxicosis / Roridins, verrucarins	*Myrothecium verrucaria*, *M. roridum* / Ryegrass, rye stubble, straw	Sheep, cattle, horses / Former USSR, New Zealand, south eastern Europe	Inflammation of many tissues, pulmonary congestion / Unthriftiness, sudden death

Section IV

Viruses, Prions and Disinfection

42 Nature, structure and taxonomy of viruses





The term 'virus' (Latin *virus*, poison) refers to members of a unique class of infectious agents which are extremely small, contain only one type of nucleic acid and are dependent on living cells for replication. The genomes of the viruses which infect animals are smaller than those of prokaryotic cells, ranging from about 2 kilobase pairs (kbp) to 200 kbp. In most viruses, the nucleic acid is present as a single molecule; in some RNA viruses the nucleic acid occurs in separate segments. Although the nucleic acid of viral genomes is usually linear, it is circular in some viruses. Genomes of DNA viruses can be single-stranded or double-stranded.

A fully assembled infective virus is termed a virion. The fundamental component of the virion is a nucleoprotein core with the ability to infect host cells and replicate in them, thus ensuring continued survival. The genome of vertebrate viruses is enclosed within a shell of proteins, called a capsid. Each subunit of the capsid is composed of a folded polypeptide chain. Collections of these subunits constitute structural units or protomers which, in turn, comprise assembly units. The term capsomer or morphological unit is used to describe features such as protrusions seen on the surface of virus particles in electron micrographs. These often correspond to groups of protein subunits arranged about a local axis of symmetry.

Characteristics of viruses which are pathogenic for animals

- Small infectious agents, ranging in size from 20 to 300 nm
- Composed of nucleic acid surrounded by a protein coat; in addition, some have envelopes
- Contain only one type of nucleic acid, either DNA or RNA
- Unlike bacteria and fungi, viruses cannot replicate on inert media; viable host cells are required for replication
- Some viruses have an affinity for particular cell types

Capsids are composed of multiples of one or more types of protein subunits. The orderly arrangement of similar protein-protein interfaces results in a symmetrical structure. Icosahedral and helical symmetries are the two types of capsid symmetry described in viruses.

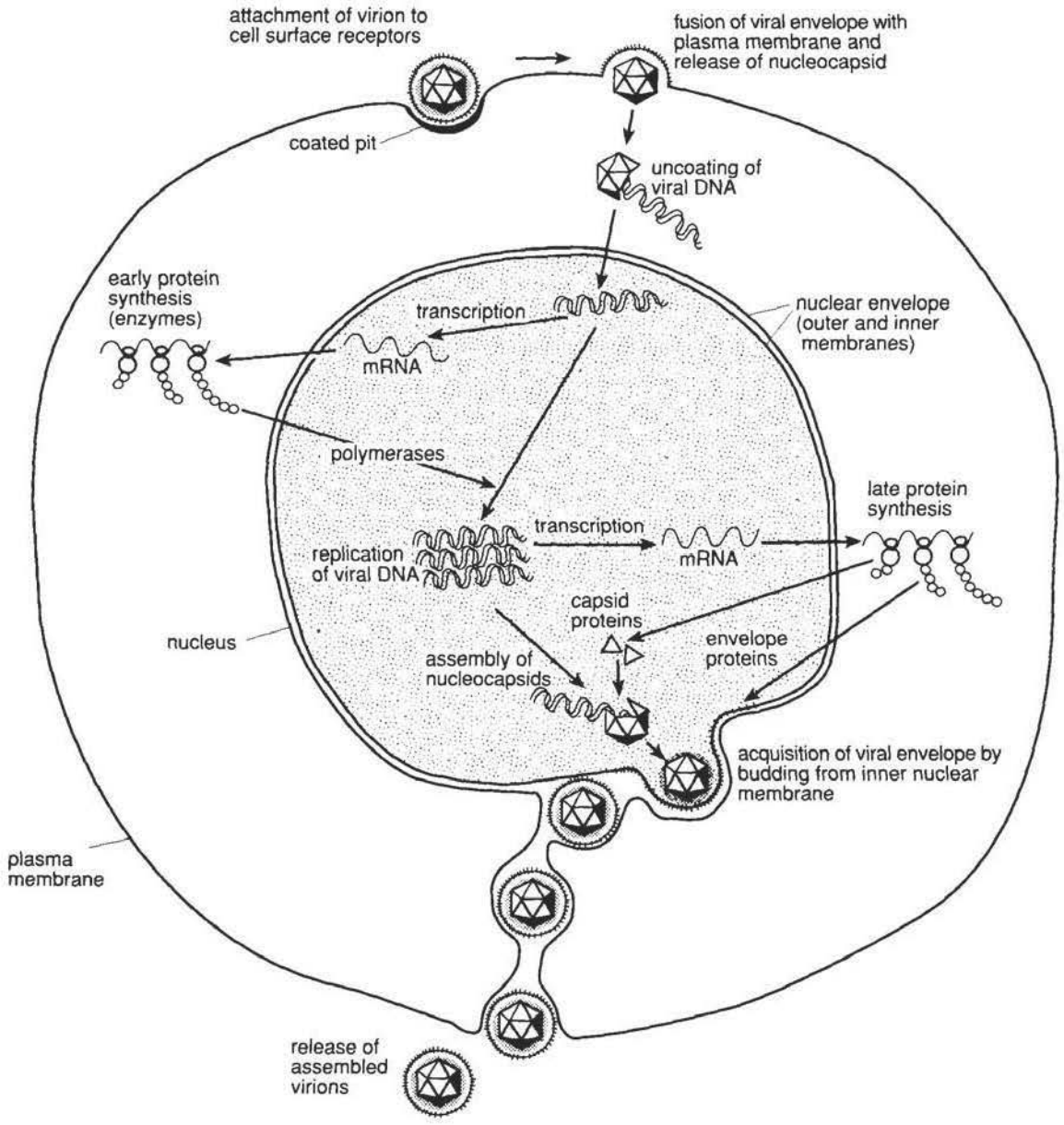
Closed shell virions, isometric viruses, have structures based on icosahedral symmetry, a structural form which offers the maximum capacity and greatest strength for a given surface area. The protective capsid of many RNA viruses is formed by the insertion of protein units between each turn of the nucleic acid helix.

In many types of viruses, the nucleocapsid is covered by an envelope composed of a lipid bilayer and associated glycoproteins. The envelope is acquired when the nucleocapsid buds through a cellular membrane. Proteins, encoded by viral nucleic acid and integrated as glycoprotein into the appropriate membrane by the compartmentalization mechanisms of the host cell, are an integral part of the viral envelope. Peplomers or spikes are knob-like projections on the envelope of certain viruses. These structures are formed from oligomers of surface glycoproteins. Taxonomically, viruses are assigned to five main hierarchical levels, namely order, family, subfamily, genus and species. A virus species is defined by a combination of multiple properties and characteristics; no single or unique property is essential for species definition. In the present scheme of virus taxonomy, the primary delineating criteria are the type and nature of the genome, the mode and site of viral replication and the structure of the virion. Currently, more than 1,500 virus species are recognized by the International Committee on Taxonomy of Viruses, with the periodic addition of new species. In addition, international specialist groups monitor large numbers of strains and subtypes. No universal definitions or formal nomenclature are recognized for strains and subtypes of virus species.

43 Replication of viruses 1

DNA virus replication

Stages in the replication of a herpes virus, an example of an enveloped double-stranded DNA virus



Unlike bacteria, which can grow on inert media, viruses can multiply only in host cells. This requirement arises from their limited genomic composition, which obliges them to utilize host cell organelles, enzymes and other macromolecules for replication. The effects of viral multiplication on host cells range from minor changes in cellular metabolism to cytolysis.

The replicative cycle of a virus can be conveniently divided into a number of stages. A virion must first attach to cell surface receptors in order to produce infection. Initial virus-cell interaction is a random event which relates to the number of virus particles present and the availability of appropriate receptor molecules. Virus-cell interaction determines both the host

Stages in replication of viruses

- Attachment to a surface receptor on a susceptible host cell
- Entry into the cell
- Uncoating of viral nucleic acid
- Replication of viral nucleic acid and synthesis of virus-encoded proteins
- Assembly of newly-formed virus particles and release from host cell

range and the tissue tropism of viral species. Viruses have evolved to the point where they can utilize a wide range of host cell surface proteins as receptors. Many of these surface molecules are highly conserved and are essential for fundamental cellular functions. Some viruses have more than one type of ligand molecule and they may bind to several cell surface receptors in sequential order during attachment. In the case of some species of virus, individual virions can detach and adsorb to another cell when infection of a particular host cell does not proceed. In the case of orthomyxoviruses and paramyxoviruses, detachment from host cells is mediated by viral neuraminidase, a receptor-destroying enzyme.

Virus uptake or penetration is an energy-dependent process which can occur in a number of ways. Receptor-mediated endocytosis occurs after virus attaches to receptors at particular sites on the plasma membrane. At these sites, which are coated internally with the protein clathrin, the virus-receptor complex is taken into the cell in specialized vesicles. The cage-like lattice formed around the vesicles by clathrin molecules breaks down after endocytosis. Acidification within the vesicles leads to degradation of viral structures. The envelopes of some viruses, such as orthomyxoviruses, rhabdoviruses and flaviviruses, fuse with the membrane of endosomes, releasing nucleocapsids directly into the cytoplasm. A second entry mechanism, which is used by some enveloped viruses including paramyxoviruses, retroviruses and herpesviruses, involves fusion of the viral envelope with the plasma membrane. This allows release of the nucleocapsid directly into the host cell cytoplasm. An additional mechanism employed by some non-enveloped viruses such as picornaviruses involves the direct introduction or translocation of viral genomes into the cytoplasm through channels in the plasma membrane.

Uncoating is the process whereby the viral genome is released in a form suitable for transcription. In the case of enveloped viruses, in which the nucleocapsid is discharged directly into the cytoplasm, transcription can usually proceed without complete uncoating. In non-enveloped viruses, uncoating is poorly understood but probably results from lysosomal proteolytic enzyme activity. In reoviruses, the genome may express all functions without complete release from the capsid. For most other non-enveloped viruses, complete uncoating occurs. Poxviruses are uncoated in two stages. The initial stage is mediated by host enzymes, with complete release of viral DNA from the core requiring virus-specified proteins. In some viruses, which replicate in the cell nucleus, uncoating may be completed at the nuclear pores.

The synthesis of viral proteins by host cells, which is the central event in replication of viruses, requires the production of viral mRNA. Those DNA viruses, which replicate in the nucleus, can avail of host cell transcriptases to synthesize viral mRNA. Other viruses utilize their own enzymes to generate mRNA. Viruses have evolved strategies which facilitate interference with the activity of cellular mRNA. Viruses direct the synthesis of either a separate mRNA for each gene or mRNA encompassing several genes. Eukaryotic cell protein-synthesizing mechanisms, however, translate only monocistronic messages. If a large precursor protein molecule is produced, cleavage into individual proteins is required and each family of viruses employs a unique strategy for this purpose.

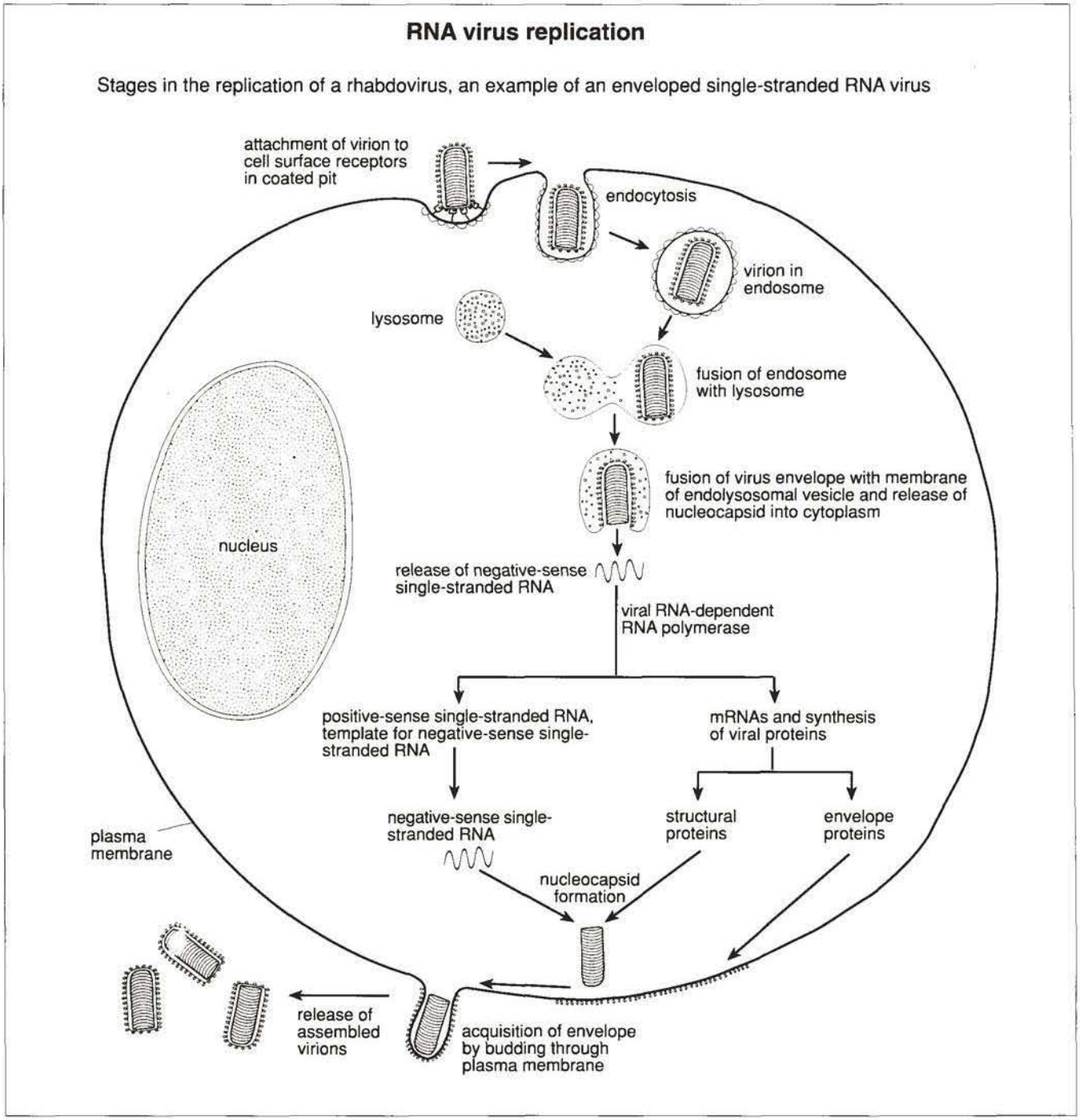
Based on the nature of the genome and the pathways of mRNA synthesis, viruses of veterinary importance can be grouped into six classes. Central to this scheme is the designation of the genome of single-stranded RNA viruses as positive-sense or negative-sense nucleic acid. In this context, the word 'sense' refers to nucleic acid polarity. The nucleic acid of positive-sense single-stranded RNA viruses is mRNA in sense and can be translated directly into virus protein.

Replication of DNA viruses

Double-stranded DNA viruses, such as herpesviruses, papovaviruses and adenoviruses, which replicate in the nucleus of the cell, have a relatively direct replication strategy. The viral DNA is transcribed by cellular DNA-dependent RNA polymerase (transcriptase) to form mRNA. In contrast, the single-stranded DNA viruses, parvoviruses and circoviruses, which also replicate in cell nuclei, utilize cellular DNA polymerase to synthesize double-stranded DNA. This is then transcribed to mRNA by cellular transcriptases. Because of this transcription requirement, the replication of parvoviruses is largely confined to rapidly-dividing cells.

A defined temporal sequence of events occurs during transcription and replication of DNA viruses. Specified genes encode for early proteins, which include the enzymes and other proteins necessary for virus replication and for suppression of the synthesis of host cell proteins. Subsequently, replication of viral nucleic acid and transcription of the genes which encode the late proteins occur. These late proteins, which are also often transcribed from newly-formed viral nucleic acid, are structural components synthesized late in the infection cycle. This temporal sequence is not clearly demonstrable in the replicative cycles of RNA viruses, in which most of the genetic information is expressed contemporaneously.

44 Replication of viruses 2



Replication of RNA viruses

Reoviruses and birnaviruses, double-stranded RNA viruses, have segmented genomes. Transcription occurs in the cytoplasm under the direction of a viral transcriptase. The negative-sense strand of each segment is transcribed to produce individual mRNA molecules. In contrast, the genomes of positive-sense, single-stranded RNA viruses can act directly as mRNA after infection. The enzymes necessary for genome replication in these viruses are produced after infection by direct translation of virion RNA. This RNA can bind directly to ribosomes and is translated to yield a single polyprotein which is then cleaved to yield both functional and structural proteins.

Because direct translation can occur, naked RNA extracted from such viruses is infectious. The positive-sense, single-stranded RNA viruses utilize a number of different synthetic pathways during replication. In togaviruses, only about two-thirds of the viral RNA is directly translated during the first round of protein synthesis. Subsequently, full-length negative-sense RNA is synthesized and, from this, a full-length positive-sense RNA destined for encapsidation and a one-third length positive-sense RNA strand are formed. The genomes of caliciviruses, coronaviruses and arteriviruses also encode for mRNA which can be full length or shorter.

Negative-sense single-stranded RNA viruses possess an RNA-dependent RNA polymerase. The naked RNA of these viruses, unlike that of the positive-sense, single-stranded RNA viruses, cannot initiate infection. After infection by the virion, the genomic RNA functions as a template for transcription of positive-sense mRNA and also for virus replication, utilizing the same polymerase. The positive-sense RNA subsequently serves as the template for synthesis of negative-sense genomic RNA. Most single-stranded, negative-sense RNA viruses replicate in the cytoplasm of the cell. Notable exceptions are orthomyxoviruses and Borna disease virus, which replicate in the nucleus. Part of the segmented genome of some members of the *Bunyaviridae* is ambisense, utilizing a mixed replication strategy with features characteristic of both positive-sense and negative-sense single-stranded RNA viruses.

The genome of retroviruses consists of positive-sense, single-stranded RNA which does not function as messenger RNA. Instead, a single-stranded DNA copy is produced by RNA-dependent DNA polymerase (reverse transcriptase) using the viral RNA as a template. As the second strand of DNA is formed, the parental RNA is removed from the RNA-DNA hybrid molecule. The double-stranded DNA is integrated into the host cell genome as a provirus. The integrated DNA provirus, which may be incorporated into cellular chromosomes at a number of sites, can be transcribed to new viral RNA.

Protein synthesis

Within the cell, the sites at which particular proteins are synthesized relate to the type and function of the protein. Membrane proteins and glycoproteins are synthesized on membrane-bound ribosomes, while soluble proteins including enzymes are synthesized on ribosomes free in the cytoplasm. Short specific amino acid sequences, known as sorting sequences, facilitate the incorporation of proteins at various cellular locations where they are required for metabolic activity. Most viral proteins undergo post-translational modification including proteolytic cleavage, phosphorylation and glycosylation. During glycosylation, sugar side-chains are added to viral proteins in a programmed manner as the proteins are being transferred from the rough endoplasmic reticulum to the Golgi apparatus. This event occurs in preparation for the final assembly of intact virions and their release from the cell.

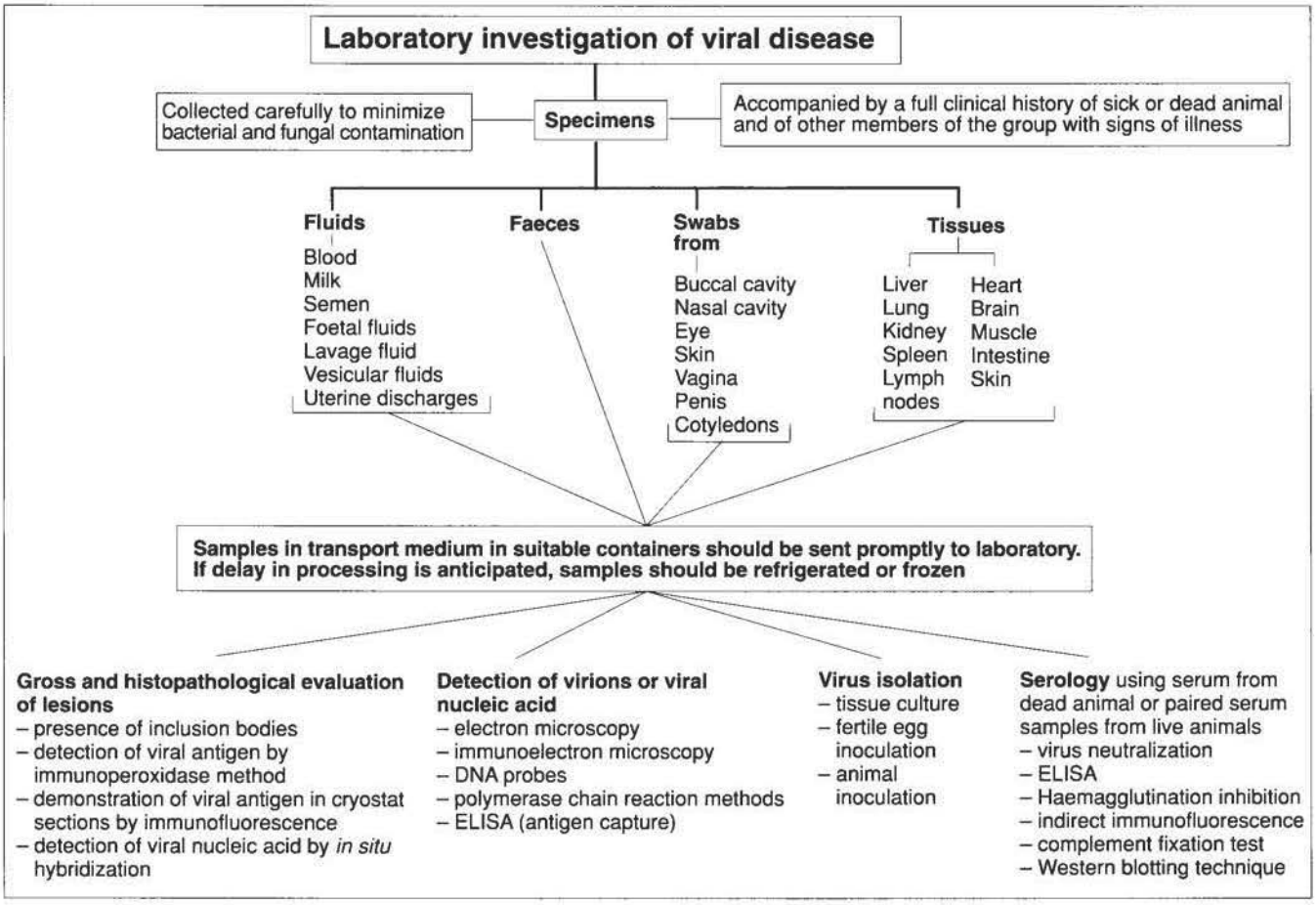
Assembly and release of virions

The mechanisms for the assembly and release of enveloped and non-enveloped viruses are distinct. Non-enveloped viruses of animals have an icosahedral structure. The structural proteins of these viruses associate spontaneously in a symmetrical and stepwise fashion to form procapsids. Subsequently, viral nucleic acid is incorporated into the procapsid. Proteolytic cleavage of specific procapsid polypeptides may be required for the final formation of infectious particles. Non-enveloped viruses are usually released following cellular disintegration. The assembly of picornaviruses and reoviruses occurs in the cytoplasm of the cell, whereas parvoviruses, adenoviruses and papovaviruses are assembled in the nucleus.

In enveloped viruses, the final step in the process of virion assembly involves acquisition of an envelope by budding from cell membranes. Prior to budding, cell membranes are modified by the insertion of virus-specified transmembrane glycoproteins, which aggregate in patches in the plasma membrane. The presence of viral glycoproteins alters the antigenic composition of infected cells which become targets for cytotoxic T lymphocytes. Togavirus nucleocapsids bind to the hydrophilic domains of the virus-specified membrane proteins, which project slightly into the cytoplasm, and become surrounded by the altered portion of membrane. The nucleocapsids of helical viruses bind to a virus-specified matrix protein which lines the cytoplasmic side of membrane patches.

Budding of viruses through the plasma membrane may not breach the integrity of the membrane and, as a result, many enveloped viruses are non-cytopathic and may be associated with persistent infections. Unlike most other enveloped viruses, togaviruses, paramyxoviruses and rhabdoviruses are cytolytic. Flaviviruses, coronaviruses, arteriviruses and bunyaviruses acquire their envelopes inside cells by budding through the membranes of the rough endoplasmic reticulum or the Golgi apparatus. These viruses are then transported in vesicles to the cell surface where the vesicle fuses with the plasma membrane releasing the virion by exocytosis. Herpesviruses, which replicate in the nucleus, are unique in that they bud through the inner lamella of the nuclear membrane and accumulate in the space between inner and outer lamellae, in the cisternae of the endoplasmic reticulum and in cytoplasmic vesicles. Release from the cell can occur either by exocytosis or by cytolysis. The assembly and release of poxviruses is a complex process taking several hours. Although replication occurs entirely in the cytoplasm of the host cell at discrete sites, termed viroplasms or 'viral factories', nuclear factors may be involved in transcription and assembly. Maturation proceeds to the formation of infectious intracellular mature virus, which can be detected following deliberate lysis of infected host cells *in vitro*. Following assembly, virus particles move out of the assembly area and become enveloped in a double membrane derived from the trans-Golgi network. At the periphery of the cell, fusion with the plasma membrane results in loss of the outer layer of the double membrane and release of extracellular enveloped virus.

45 Laboratory diagnosis of viral disease



Many viral diseases of animals can be diagnosed on the basis of clinical signs together with postmortem findings and histopathological findings. However, confirmation of the involvement of specific viral pathogens often requires special laboratory procedures. Surveillance for particular viruses is an important aspect of the management of valuable animals such as bulls used for artificial insemination, and stallions, which have the potential to spread infection to many other animals. As part of international trade regulations, certification of freedom from certain viral diseases must accompany animals exported to countries in which the diseases are exotic. Moreover, rapid and accurate laboratory confirmation of exotic viral diseases, including those with zoonotic potential, is essential for the successful implementation of eradication policies and for the protection of human health. Surveillance of animal populations for new or emerging viral diseases is an important responsibility of national veterinary services.

More than 200 major viral diseases of veterinary importance affect animal species. Because of the considerable resources required for the provision of comprehensive diagnostic services in virology, national diagnostic services usually concentrate on those diseases prevalent in a country. Moreover, laboratories often provide a diagnostic service for particular animal species. Special laboratory containment facilities are mandatory for some viruses which cause highly contagious diseases such as foot-and-mouth disease. The *Office International des Epizooties* (OIE) in Paris monitors and publishes details of significant animal disease outbreaks worldwide. This work is possible only through international cooperation and a network of laboratories dealing with viral diseases of international importance.

Collection, preservation and transportation of samples

Ideally, specimens for laboratory examination should be collected as early as possible from affected animals before secondary bacterial or fungal infections become established. It is advisable to collect samples from apparently normal in-contact animals because some of these animals may be actively shedding virus. The specimens selected for examination should

relate to the clinical signs or to lesion distribution at post-mortem. Swabs from the oropharynx or nasopharyngeal aspirates are suitable specimens for investigation of respiratory diseases. In enteric viral diseases, large numbers of virus particles are shed in faeces. In those diseases characterized by viraemia, virus may be demonstrable in cells of the buffy coat.

Preservation of the infectivity or antigenicity of viruses may be required for particular tests. As many viruses are labile, specimens for virus isolation should be collected into transport medium, refrigerated and transmitted to the laboratory without delay. Samples should be frozen at –70°C if delay in delivery is anticipated. Freezing in a domestic freezer at –20°C decreases the infectivity of most viruses. Transport medium consists of buffered isotonic saline containing a high concentration of protein, such as bovine albumin or foetal calf serum, which prolongs virus survival. Antibiotics and antifungal drugs are added in order to inhibit the growth of contaminants. Samples for electron microscopy, in which the demonstration of virion morphology is the primary objective, require less exacting conditions for storage and transportation. Air-dried smears for fluorescent antibody (FA) staining should be fixed in either acetone or methanol for up to 10 minutes in order to preserve viral antigens. This fixation process allows penetration of FA conjugates into cells. A similar fixation procedure is required for cryostat sections of frozen tissues prior to FA staining. Formalin-fixed tissue samples embedded in paraffin wax can be stored for many years and used to demonstrate the presence of viral antigen by immunohistochemical techniques.

Guidance from clinicians regarding the possible aetiology of the disease under investigation is essential for deriving maximum benefit from laboratory tests. This requires an accurate assessment of the history and clinical signs, together with a tentative clinical diagnosis. In some instances, postmortem and histopathological examination of tissues may be sufficient for diagnostic purposes, particularly if specific inclusion bodies are found in infected tissues.

Detection of virus, viral antigens or nucleic acid

The presence of virus in tissues can be confirmed by isolation of live virus, by demonstration of virus particles or viral antigen and by detecting viral nucleic acid. Virus isolation using cell culture, fertile eggs or experimental animals is the standard against which other diagnostic methods are usually compared. A monolayer composed of a particular cell type cannot be expected to support the growth of the many viruses which cause animal diseases. Laboratories usually have a limited range of cell lines most often used for virus isolation and appropriate to the range of samples received. Embryonated eggs are widely used for the isolation of influenza A virus and avian viruses. Virus isolation is a sensitive procedure when cultural conditions are optimal for a particular virus, and this method also generates a supply of virus for further studies. However, it is labour-intensive, slow and expensive. A number of blind passages may be required before a virus becomes adapted to a particular cell line and, as a consequence, a test result may not be available for some weeks. Because some viruses do not produce a cytopathic effect, additional detection procedures such as haemadsorption and FA staining may be needed to demonstrate their presence in cell cultures. Even when a virus produces a pronounced cytopathic effect, additional tests are often required for definitive identification.

The sensitivity and versatility of methods for the detection of viral nucleic acids have greatly improved in recent years and these are now becoming the methods of choice for viral identification. These methods are particularly valuable when dealing with viruses which are either difficult to grow or cannot be grown *in vitro*. They are useful for detecting latent infections in which infectious virus is absent and also for specimens containing inactivated virus. Cloned viral DNA is available for the probing of samples and tissues by nucleic acid hybridization. This technique, however, has been largely replaced in recent years by the polymerase chain reaction which has the advantage of amplifying the target gene sequences.

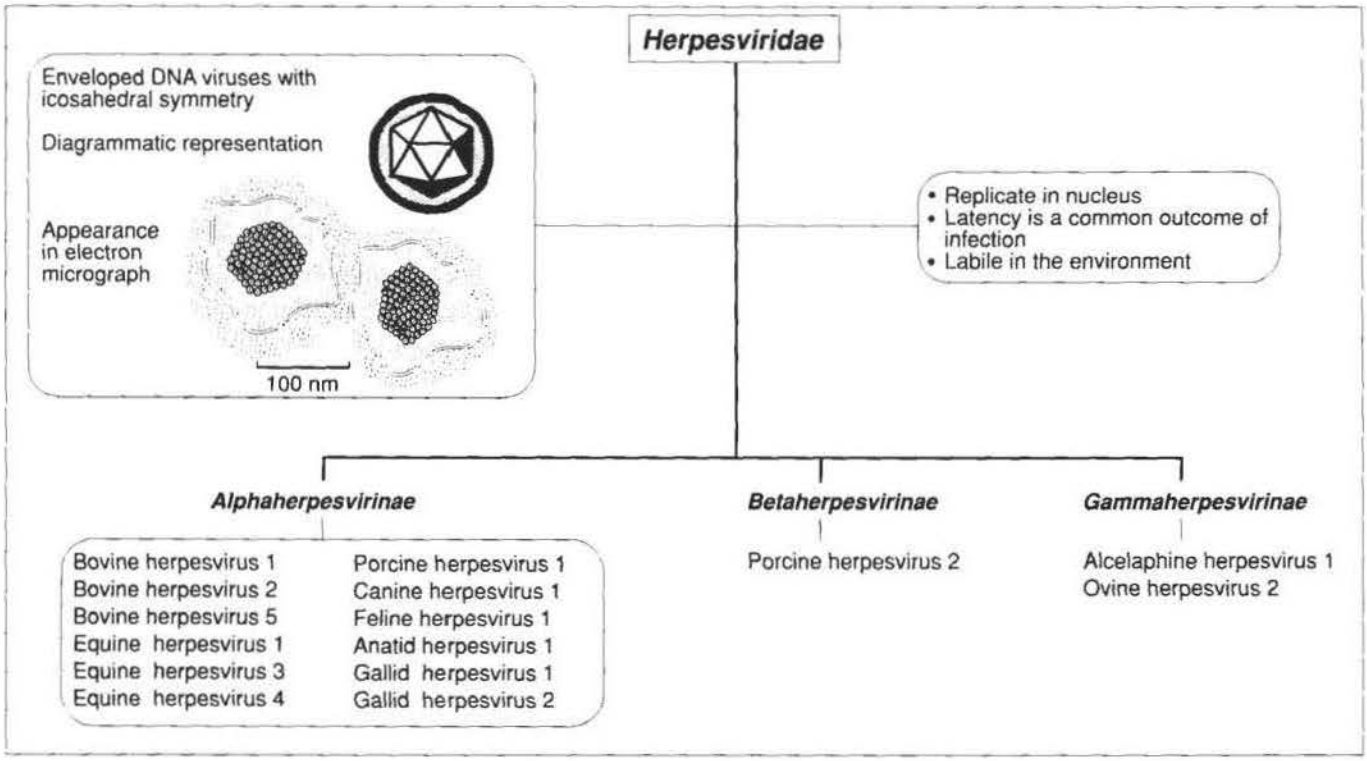
Diagnostic serology

Serological procedures can be used for the retrospective diagnosis of viral diseases and for epidemiological surveys. These procedures can be automated and diagnostic reagents for many viral pathogens are commercially available. Single blood samples from animals in susceptible populations suffice for establishing the prevalence of a disease. When using serological procedures for the diagnosis of endemic disease in flocks or herds, paired serum samples taken at an interval of at least three weeks, are required to demonstrate rising antibody titres. Initial samples should be collected when clinical signs are first evident and the second group during convalescence. A single blood sample may be adequate for diagnosis if reagents are available for demonstrating IgM antibodies, which are indicative of a primary immune response. Difficulties with the interpretation of serological tests may arise due to cross-reactions with antigenically related viruses. In young animals, passively acquired maternal antibodies, which may persist for several months, can lead to difficulty in interpretating results.

Interpretation of test results

Because false-positive and false-negative results can occur in many test procedures, inclusion of positive and negative controls is essential. The sensitivity and specificity of a particular diagnostic test should be established. The sensitivity of a diagnostic test, expressed as a percentage, is the number of animals identified as positive out of the total number of animals with the disease. The specificity of a test is the percentage of uninfected animals in which the result is negative. In order to detect all animals with an important viral infection, a test with high sensitivity is required. For laboratory confirmation of a viral infection in an individual animal, a test with high specificity is essential.

46 *Herpesviridae* 1



The family *Herpesviridae* contains more than 100 viruses. Fish, amphibians, reptiles, birds and mammals including humans are susceptible to herpesvirus infection. These viruses are of special importance because of their widespread occurrence, their evolutionary diversity and their involvement in many important diseases of domestic animals and humans. The name, herpesvirus (Greek *herpein*, to creep), refers to the sequential appearance and local extension of lesions in human infection. Herpesviruses are enveloped and range from 120 nm to 200 nm in diameter. They contain double-stranded DNA within an icosahedral capsid. Herpesviruses enter cells by fusing with the plasma membrane. Replication occurs in the cell nucleus. The envelope, which derives from the nuclear membrane of the host cell, incorporates at least eight viral encoded glycoproteins. Enveloped virions accumulate in the endoplasmic reticulum prior to final processing of the glycoproteins in the Golgi apparatus and release by exocytosis. Active infection results in cell death. Intranuclear inclusions are characteristic of herpesvirus infections. Extension of viral infection occurs through points of cell contact without exposure of virus to neutralizing antibodies in blood or interstitial fluids. Protective antibody responses are usually directed against the envelope glycoproteins. Herpesvirus virions, which are fragile and sensitive to detergents and lipid solvents, are unstable in the environment.

The family is divided into three subfamilies comprising nine genera. Alphaherpesviruses replicate and spread rapidly, destroying host cells and often establishing latent infections in sensory ganglia. Betaherpesviruses, which replicate and spread slowly, cause infected cells to enlarge, hence their common name, cytomegaloviruses. They may become latent in secretory glands and lymphoreticular cells. Gammaherpesviruses, which infect T or B lymphocytes, can produce latent infections in these cells. When lymphocytes become infected, there is minimal expression of viral antigen. Some gammaherpesvirus species also replicate in epithelial and fibroblastic cells, causing cytolysis. A number of gammaherpesviruses are implicated in neoplastic transformation of lymphocytes.

Clinical infections

Herpesviruses establish life-long infections with periodic reactivation resulting in bouts of clinical disease. Shedding of virus may be periodic or continuous. During latency, the episomal viral genome becomes circular and gene expression is limited. Reactivation of infection is associated with various stress factors including transportation, adverse weather conditions, overcrowding and intercurrent infection. Natural infections with particular herpesviruses are usually restricted to defined host species. Because these viruses are highly adapted to their natural hosts, infections may be inapparent or mild. However, in very young or immunosuppressed animals, infection can be life-threatening.

Herpesviruses can cause respiratory, genital, mammary and CNS diseases in cattle (Table 46.1). Aujeszky's disease, which

Table 46.1 Herpesvirus infections of ruminants.

Virus	Genus	Comments
Bovine herpesvirus 1	*Varicellovirus*	Causes respiratory (infectious bovine rhinotracheitis) and genital (infectious pustular vulvovaginitis, balanoposthitis) infections. Occurs worldwide
Bovine herpesvirus 2	*Simplexvirus*	Causes ulcerative mammillitis in temperate regions and pseudo-lumpy-skin disease in tropical and subtropical regions
Bovine herpesvirus 5	*Varicellovirus*	Causes encephalitis in calves; described in several countries
Ovine herpesvirus 2	*Rhadinovirus*	Causes subclinical infection in sheep and goats worldwide. Causes malignant catarrhal fever in cattle and in some wild ruminants
Alcelaphine herpesvirus 1	*Rhadinovirus*	Causes subclinical infection in wildebeest in Africa and in zoos. Causes malignant catarrhal fever in cattle, deer and in other susceptible ruminants

affects pigs and other domestic species, is the major porcine herpesvirus infection (Table 46.2).

Infectious bovine rhinotracheitis and pustular vulvovaginitis

Infection with bovine herpesvirus 1 (BHV-1) is an important cause of losses in cattle worldwide. It is associated with several clinical conditions including infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, balanoposthitis, conjunctivitis and generalized disease in newborn calves.

The virus is usually acquired through aerosols. Replication occurs in the mucous membranes of the upper respiratory tract and large amounts of virus are shed in nasal secretions. Virus also enters local nerve cell endings and is transported intraaxonally to the trigeminal ganglion, where it remains latent. In most instances, infection is contained within two weeks by a strong immune response. However, tissue necrosis may facilitate secondary bacterial infection, with severe systemic effects and, possibly, death. Rarely, a lymphocyte-associated viraemia in pregnant cows may produce foetal infection and abortion.

Following genital infection, virus replicates in the mucosa of the vagina or prepuce, and latent infection may become established in the sacral ganglia. Focal necrotic lesions on genital mucosae may eventually coalesce to form large ulcers.

In outbreaks of disease, either the respiratory or the genital form usually predominates. Swabs collected from the nares and genitalia of several affected animals during the early acute phase of the disease are suitable for virus isolation or the production of smears for the rapid demonstration of viral antigen using immunofluorescence. Inactivated, subunit and modified live vaccines are available for control. Vaccination reduces the severity of clinical signs but may not prevent infection.

Aujeszky's disease

This disease, also called pseudorabies, is caused by porcine herpesvirus 1. The pig is the natural host of the virus and infection is endemic in the pig populations of many countries. Aujeszky's disease virus (ADV) is shed in oronasal secretions, milk and semen. Transmission usually occurs by nose-to-nose contact or by aerosols. Following infection, the virus replicates in the epithelium of the nasopharynx and tonsils. Virus spreads to regional lymph nodes and to the CNS along axons of the cranial nerves. Virulent strains produce a brief viraemia and become widely distributed around the body, particularly in the respiratory tract. Transplacental transfer results in generalized infection of foetuses. Latency occurs in a high percentage of infected animals, with virus localized in the trigeminal ganglia and tonsils.

The age and susceptibility of infected pigs and the virulence of the infecting strain influence the severity of the clinical signs. Young pigs are most severely affected; mortality may approach 100% in suckling piglets. Neurological signs predominate in young pigs. Mortality is much lower in weaned pigs, although neurological and respiratory signs are often present. Infection in sows may result in resorption of foetuses, abortion or stillbirths. In herds with endemic ADV infection, neonatal animals are protected by maternally-derived antibody.

Specimens of brain, spleen and lung from acutely affected animals are suitable for virus isolation, while cryostat sections of tonsil or brain are suitable for detection of viral antigen by immunofluorescence. If used strategically, vaccination can prevent the development of clinical disease. Modified live, inactivated and gene-deleted marker vaccines are available.

Disease in other domestic animals occurs sporadically and is characterized by neurological signs resembling those of rabies. Marked pruritis is a feature of the disease in species other than pigs. The clinical course is short, with most affected animals dying within a few days.

Table 46.2 Herpesvirus infections of pigs.

Virus	Genus	Comments
Porcine herpesvirus 1 (Aujeszky's disease virus)	*Varicellovirus*	Causes Aujeszky's disease (pseudorabies) primarily in pigs. Encephalitis, pneumonia and abortion are features of the disease. In many species other than pigs, pseudorabies manifests as a neurological disease with marked pruritis. Occurs worldwide
Porcine herpesvirus 2	Unassigned	Caŭses disease of the upper respiratory tract in young pigs (inclusion body rhinitis)

47 *Herpesviridae 2*

Equine herpesvirus infections are presented in Table 47.1; those of domestic carnivores are listed in Table 47.2 and those of birds in Table 47.3.

Equine rhinopneumonitis and equine herpesvirus abortion

Infection with equine herpesvirus 1 (EHV-1) is associated with respiratory disease, abortion, fatal generalized disease in neonatal foals and encephalomyelitis. Close contact facilitates transmission of these fragile viruses. Transmission usually occurs by the respiratory route following contact with infected nasal secretions, aborted foetuses, placentae or uterine fluids. The viruses replicate initially in the upper respiratory tract and regional lymph nodes with spread, in some cases, to the lower respiratory tract and lungs.

Abortion caused by EHV-1 occurs several weeks or months after exposure, usually during the last four months of gestation. Equine herpesvirus 1 has a predilection for vascular endothelium. Vasculitis and thrombosis in the placenta, along with transplacental infection of the foetus, results in abortion. Infected mares rarely abort during subsequent pregnancies and their fertility is unaffected. Infection close to term may result in the birth of an infected foal which usually dies due to interstitial pneumonia and viral damage in other tissues, sometimes complicated by secondary bacterial infection. Vasculitis and thrombosis in EHV-1 infection may affect the CNS, especially the spinal cord. Neurological changes appear to be related to infection with particular strains of EHV-1. Although neurological signs associated with EHV-1 infection are relatively uncommon, they may present in several horses during an outbreak of abortion or respiratory disease on a farm. The signs range from slight incoordination to paralysis, recumbency and death.

Respiratory disease caused by equine herpesvirus 4 (EHV-4) occurs in foals over two months of age, in weanlings and in yearlings. Following an incubation period of two to ten days, there are signs of fever, pharyngitis and serous nasal discharge. Secondary bacterial infection is common, giving rise to mucopurulent nasal discharge, coughing and, in some cases, bronchopneumonia. Outbreaks of respiratory disease caused by EHV-1 are less common.

Virus isolation and identification are used routinely for the laboratory confirmation of herpesvirus infection in horses. Viral antigen may be demonstrated in cryostat sections of lung, liver and spleen from aborted foetuses using immunofluorescence.

Effective management practices and vaccination are essential for control. Animals returning from sales, races or other events should be segregated for up to four weeks. On large stud farms, horses should be kept in small, physically-separated groups. Modified live and inactivated virus vaccines are commercially available. As vaccination is not considered to be fully protective, frequent boosters are recommended. Vaccination appears to reduce the severity of clinical signs and to decrease the likelihood of abortion.

Table 47.1 Herpesvirus infections of horses.

Virus	Genus	Comments
Equine herpesvirus 1	*Varicellovirus*	Causes abortion, respiratory disease, neonatal infection and neurological disease. Occurs worldwide
Equine herpesvirus 3	*Varicellovirus*	Causes mild venereal infection in both mares and stallions
Equine herpesvirus 4	*Varicellovirus*	Causes rhinopneumonitis in young horses and sporadic abortion. Occurs worldwide

Canine herpesvirus infection

Infection in domestic and wild *Canidae* caused by canine herpesvirus 1 (CHV-1) is common worldwide. Clinical disease caused by the virus, which is characterized by high mortality following generalized infection in neonatal pups, is uncommon.

Infection usually occurs by the oronasal route following direct contact between infected and susceptible animals. During periods of stress, latent infections may be reactivated, with shedding of virus. The sites of latency include sensory ganglia. Virus is shed in oronasal and vaginal secretions. Newborn pups, which can acquire infection either during parturition or *in utero*, may transmit infection to littermates.

Following infection, CHV-1 replicates in the nasal mucosa, pharynx and tonsils. The virus replicates most effectively at temperatures below normal adult body temperature. Because the hypothalamic regulatory centre is not fully operational in pups under four weeks of age, they are particularly dependent on ambient temperature and maternal contact for maintenance of normal body temperature. A cell-associated viraemia and

Table 47.2 Herpesvirus infections of domestic carnivores.

Virus	Genus	Comments
Canine herpesvirus 1	*Varicellovirus*	Causes a fatal generalized infection in neonatal pups
Feline herpesvirus 1	*Varicellovirus*	Causes feline viral rhinotracheitis in young cats

widespread viral replication in visceral organs can occur in infected neonatal animals with subnormal body temperatures. Affected pups stop suckling, show signs of abdominal pain, whine incessantly and die within days. Morbidity and mortality rates in affected litters are high. Bitches whose pups are affected tend to produce healthy litters subsequently.

Diagnostically significant postmortem findings include focal areas of necrosis and haemorrhage, particularly in the kidneys. Intranuclear inclusions are usually present. Virus isolation can be carried out in canine cell lines from fresh specimens of liver, kidney, lung and spleen. No commercial vaccine is available. Affected bitches and their litters should be isolated to prevent infection of other whelping bitches.

Feline viral rhinotracheitis

This acute upper respiratory tract infection of young cats is caused by feline herpesvirus 1 (FHV-1). The virus, which is distributed worldwide, accounts for about 40% of respiratory infections in cats.

Close contact is required for transmission. Most recovered cats are latently infected. Reactivation with virus replication and shedding is particularly associated with periods of stress such as parturition, lactation or change of housing. Initially, FHV-1 replicates in oronasal or conjunctival tissues before infecting the epithelium of the upper respiratory tract. Secondary bacterial infections, which commonly occur, exacerbate the clinical signs. Young cats display signs of acute upper respiratory tract infection including fever, sneezing, inappetence, hypersalivation, conjunctivitis and oculonasal discharge. In more severe disease, pneumonia or ulcerative keratitis may be evident. The mortality rate is low except in young or immunosuppressed animals.

Clinical differentiation of feline viral rhinotracheitis from feline calicivirus infection is difficult. Virus can be isolated in feline cell lines from oropharyngeal or conjunctival swabs. Specific viral antigen can be demonstrated in acetone-fixed nasal and conjunctival smears using immunofluorescence. Good husbandry practices and disease control procedures should be implemented in catteries in conjunction with regular vaccination schedules to minimize the impact of clinical disease. Commercial vaccine preparations also contain feline calicivirus. The protection provided by vaccination is incomplete as vaccinated cats can become infected but clinical signs tend to be much reduced.

Marek's disease

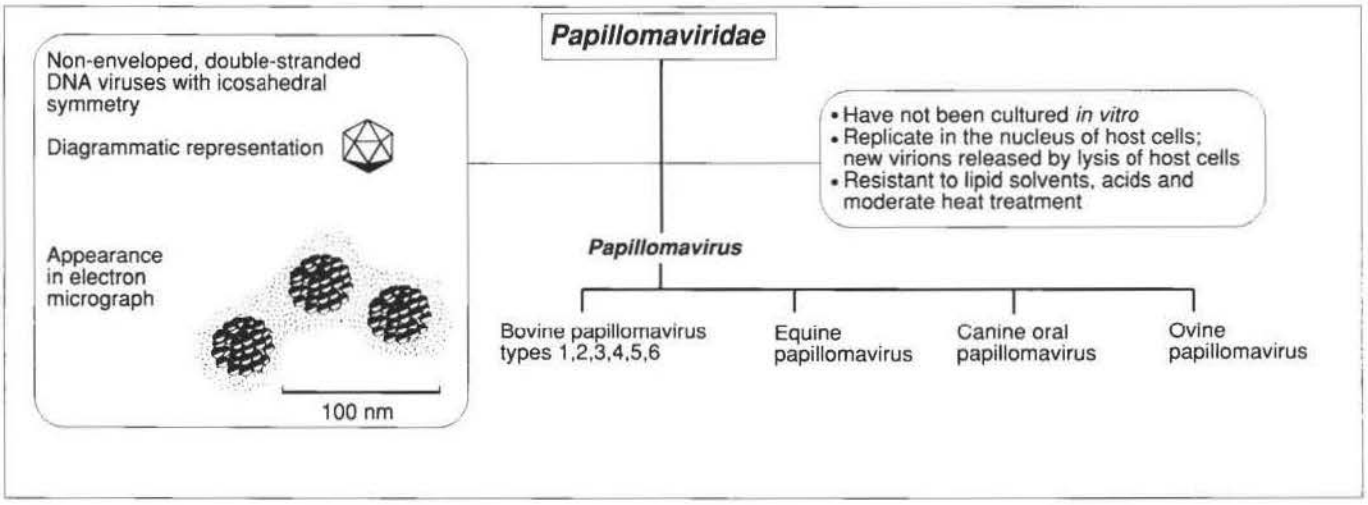
This contagious lymphoproliferative disease of chickens is caused by gallid herpesvirus 2 (Marek's disease virus), which is cell-associated and oncogenic. The disease, which is of major economic significance in the poultry industry, occurs worldwide. Productive replication with release of infective virus occurs only in the epithelium of the feather follicle. Cell-free virus is released from the follicles along with desquamated cells. This dander can remain infective for several months in dust and litter in poultry houses. Infected birds remain carriers for life and their chicks, which are protected initially by maternally-derived antibody, acquire infection within a few weeks, usually by the respiratory route. In addition to the virulence of the infecting strain of herpesvirus, host factors which contribute to the severity of the disease include the sex, age at the time of infection and genotype. Female birds are more susceptible than male birds, while resistance to the development of disease increases with age. The bird's genotype influences the susceptibility of T lymphocytes to transformation and development of lymphoid tumours. Birds between 12 and 24 weeks of age are most commonly affected. Clinically, Marek's disease presents as partial or complete paralysis of the legs and wings.

The diagnosis of Marek's disease is based on clinical signs and pathological findings. Differentiation from lymphoid leukosis is based on the age of affected birds, the incidence of clinical cases and the histopathological findings. The use of appropriate management strategies, genetically resistant stock and vaccination have reduced losses from Marek's disease. Disinfection, all-in/all-out policies, and rearing young chicks away from older birds for the first two or three months of life reduce exposure to infection, decreasing the likelihood of serious disease. A range of modified live vaccines are commercially available. Although a single dose of virus injected into day-old chicks provides good lifelong protection, it does not prevent superinfection with virulent field viruses. Novel vaccines, based on recombinant DNA technology, are being developed.

Table 47.3 Herpesvirus infections of birds.

Virus	Genus	Comments
Gallid herpesvirus 1	Infectious laryngotracheitis-like viruses	Causes infectious laryngotracheitis. Present in many countries
Gallid herpesvirus 2 (Marek's disease virus)	Marek's disease-like viruses	Causes Marek's disease, a lymphoproliferative condition in 12 to 24 week old chickens. Occurs worldwide
Anatid herpesvirus 1	Unassigned	Causes acute disease in ducks, geese and swans characterized by oculonasal discharge, diarrhoea and high mortality. Occurs worldwide

48 *Papillomaviridae*



Formerly, papillomaviruses were grouped with polyomaviruses in the family *Papovaviridae*. Infections with polyomaviruses are of minor veterinary significance. Papillomaviruses usually infect the basal cells of squamous epithelium as a result of minute abrasions. Infected cells proliferate and differentiation is delayed. Viral gene expression is restricted during this proliferative phase. Full gene expression results in the production of viral capsids only after cellular differentiation begins in the upper layers of the epithelium. The release of virus occurs during desquamation of infected cells from the surface of lesions.

Clinical infections

The epitheliotropic, host-specific papillomaviruses (Latin *papilloma*, nipple) cause proliferative lesions (warts) in many mammalian and avian species. Although they have not been grown in cell culture, the DNA sequences of several papillomaviruses have been elucidated, allowing specific detection in lesions. In infected cells, the viral DNA is usually episomal. Papillomaviruses are used experimentally for inserting foreign DNA into cultured cells.

Each papillomavirus tends to be host-specific and to produce proliferative lesions in specific anatomical sites. Although infections with papillomaviruses occur in many animal species, only those which affect cattle, horses and dogs are of clinical significance. Infections are often persistent and usually established early in life. Lesions are most commonly observed in young animals and usually regress spontaneously after weeks or months. Regression is attributed to the development of cell-mediated immunity. Typical papillomas are composed of finger-like projections of proliferating epithelium supported by a thin core of mature fibrous tissue. In fibropapillomas, the fibrous tissue component predominates. More than 80 types have been identified in humans, while in cattle six types are recognized. Individual types of virus share less than 50% sequence homology and exhibit differences in reciprocal serological assays. Progression of papillomas to malignant tumours has been documented in humans, cattle and rabbits.

Bovine cutaneous papillomatosis

Fibropapillomas arising from infection with BPV types 1 or 2 are often found on the head and neck of cattle under two years of age. Spontaneous regression of the lesions generally occurs within 1 year. Cutaneous papillomas caused by BPV-3 tend to persist. Because infection with BPV is usually self-limiting, treatment is seldom required. Teat fibropapillomas, associated with BPV-5 infection, have smooth surfaces and are described as 'rice grain' type. In contrast, 'frond' type teat papillomas arise from infection with BPV-6. Surgical removal of large lesions on teats may be necessary because of interference with milking.

Bovine alimentary papilloma-carcinoma complex

Papillomas of the oesophagus, rumen and reticulum are associated with BPV-4 infection. The lesions, which are often solitary and relatively small, are found incidentally at post-mortem examination. Epidemiological and experimental studies have demonstrated that there is an increased frequency in the occurrence of malignant transformation of virus-induced alimentary papillomas to squamous cell carcinomas when animals are ingesting bracken fern. Such malignant lesions may cause difficulty in swallowing, ruminal tympany and loss of condition. Nodular fibropapillomas caused by BPV-2, which

are occasionally found in similar upper alimentary tract locations, do not appear to become malignant.

Enzootic haematuria

Enzootic haematuria is encountered worldwide in cattle on poor pastures with abundant bracken fern growth. The haemorrhage originates from tumours in the bladder wall. Individual neoplastic lesions derive from either epithelial or mesenchymal tissues. Experimental studies suggest that BPV-2 and toxic compounds from bracken contribute to oncogenesis. It is probable that immunosuppression following ingestion of bracken may allow activation of latent BPV-2 in bladder tissues and this effect, together with the action of carcinogens also present in bracken, is responsible for the induction and progression of neoplastic lesions.

Equine papillomatosis

Papillomas are commonly encountered in horses between one and three years of age. Two types of equine papillomavirus have been identified based on DNA studies. Type 1 is associated with papillomas on the muzzle and legs while type 2 is associated with papillomas of the genital tract. Spread may occur by direct or indirect contact. The lesions usually regress spontaneously after several months and recovered animals are immune to reinfection.

Equine sarcoid

The equine sarcoid, a locally invasive fibroblastic skin tumour, is the most common neoplasm of horses, donkeys and mules. Bovine papillomavirus types 1 and 2 or closely related viruses are implicated in sarcoid development. Experimental inoculation with these viruses results in fibromatous lesions which resemble sarcoids but which regress spontaneously. Viral DNA with a high degree of homology to BPV has been identified in tissue from sarcoids using both *in situ* hybridization and PCR.

Lesions usually develop in horses between three and six years of age. Multiple cases can occur in families or groups of horses in close proximity. However, the incidence of equine sarcoid (estimated at 0.5% to 2%), is comparatively low for a viral disease indicating that the horse may be a non-permissive host.

Sarcoids can occur on any part of the body, either singly or in clusters. The most commonly affected sites are the head, ventral abdomen and limbs. They are highly variable in appearance but can be arbitrarily categorized as verrucous or fibroblastic. Clinical diagnosis should be confirmed histologically. Surgical removal is the usual form of treatment. Recurrence is common following conventional surgery and cryosurgery is more successful. Radiation therapy, CO_2 laser surgery and chemotherapy have also been used with varying degrees of success. Immunotherapy, aimed at stimulating cell-mediated immunity, may be effective in some cases. This involves intralesional injection of BCG or cell wall extract of *Mycobacterium bovis* into horses previously sensitized to tuberculoprotein.

Canine oral papillomatosis

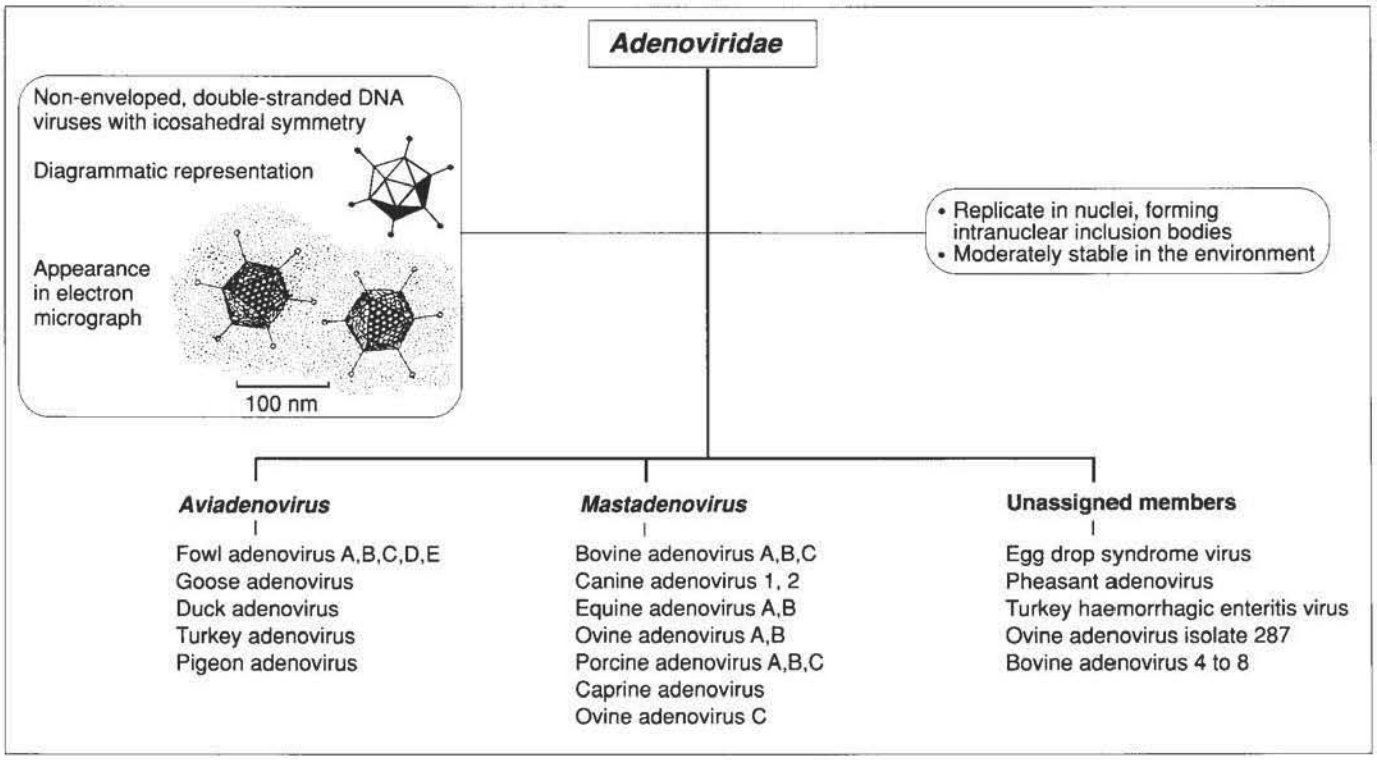
Multiple transmissible papillomas in the oropharyngeal region of dogs are often encountered. The disease, which is caused by canine oral papillomavirus, is common in young dogs and is readily transmitted. While the aetiology of this oral condition is well established, the cause of papillomas occurring at other sites in the dog is uncertain.

Canine oral papillomavirus is transmitted by direct and indirect contact. The incubation period is up to 8 weeks. Lesions are usually multiple and although generally confined to the oral mucosa, are sometimes found on the conjunctiva, eyelids and muzzle. The papillomas initially appear as smooth, white, raised lesions but later become rough and cauliflower-like. Spread may occur inside the oral cavity. There is spontaneous regression within months. Surgical removal is generally unnecessary unless the papillomas persist or cause physical discomfort. Inactivated vaccines have been used but do not appear to be effective. Live, unattenuated vaccines, which are effective, may produce neoplastic lesions at the injection site.

Diagnosis

The clinical appearance of papillomas (warts) is distinctive. Laboratory confirmation is not usually required for papillomatous lesions. Histopathological examination may be required to determine the nature of some lesions, especially equine sarcoids. Electron microscopic examination of specimens from the epidermis may reveal characteristic virus particles. Hybridization assays and PCR methods are available for the detection of papillomavirus DNA, but are not used routinely. Isolates can be typed by extraction of DNA and restriction endonuclease analysis or by Southern blotting.

49 *Adenoviridae*



Adenoviruses (Greek *adenos*, gland) were first isolated from explant cultures of human adenoids. Mammalian adenoviruses, assigned to the genus *Mastadenovirus*, infect mammals only, share a common antigen and are serologically distinct from those that infect birds. Serogroups and serotypes are defined on the basis of neutralization assays.

Clinical infections

Adenoviruses have a natural host range generally confined to a single species or to closely related species. Infection is common in animals and humans. Adenoviruses of veterinary importance are presented in Table 49.1. Adenovirus infections can be particularly severe in dogs and domestic fowl. In other domestic mammals, adenovirus infections are associated occasionally with enteric or respiratory problems. Avian adenoviruses are distributed worldwide and infection is extremely common in poultry flocks. Most of these infections are either subclinical or associated with relatively mild disease. However, severe disease may follow infection with egg-drop syndrome virus and turkey haemorrhagic enteritis virus.

Infectious canine hepatitis

This worldwide, generalized viral disease of dogs principally affects the liver and vascular endothelium. Infectious canine hepatitis has become relatively uncommon because of the widespread use of effective vaccines. Although dogs are the most commonly affected species, foxes, wolves, coyotes, skunks and bears are also susceptible. Transmission can occur following ingestion of urine, faeces or saliva from infected animals. The immune response usually eliminates virus from host tissues by 14 days after initial infection. However, virus may persist in the kidneys and, in some instances may be excreted in urine for more than six months.

Following ingestion, canine adenovirus 1 (CAV-1) localizes in the tonsils and Peyer's patches. As viraemia develops, replication in vascular endothelium results in rapid distribution of virus throughout the body. Virus replication also occurs in the parenchymal cells of the liver and kidneys. Clinical recovery in most dogs coincides with the production of neutralizing antibodies about ten days after infection. Glomerulonephritis, corneal oedema and anterior uveitis, attributable to immune complex deposition, may develop in some infected animals.

The incubation period is up to seven days. Dogs of all ages are susceptible and subclinical infection is common. Clinical disease is most frequently encountered in young dogs. The mortality rate ranges from 10% to 30% in mature dogs and up to 100% in young pups. In peracute disease, death occurs so rapidly that poisoning may be suspected. In acute disease, affected dogs present with fever, depression, anorexia, increased thirst, vomiting and diarrhoea. Abdominal palpation may elicit pain and, although hepatomegaly may be detected, jaundice is uncommon. Corneal opacity, either unilateral or bilateral,

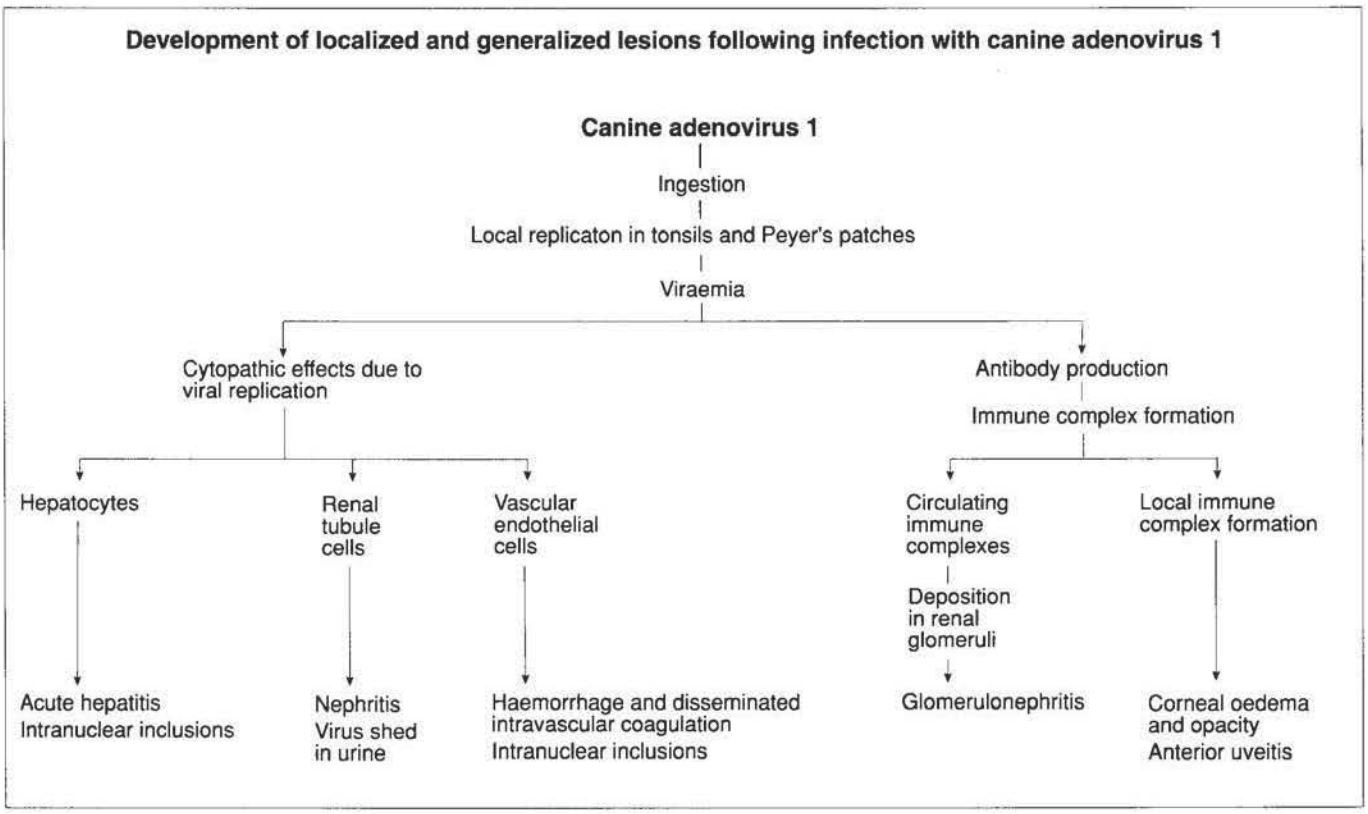


Table 49.1 Adenoviruses of veterinary importance.

Virus	Comments
Canine adenovirus 1	Causes infectious canine hepatitis, with lesions arising from direct cytopathic effects and immune complex formation
Canine adenovirus 2	Involved in infectious tracheobronchitis (kennel cough), a highly contagious respiratory disease
Equine adenovirus A	Usually a subclinical or mild respiratory infection; associated with pneumonia in Arabian foals with combined immunodeficiency disease
Bovine adenoviruses	Associated with occasional outbreaks of respiratory and enteric disease
Ovine adenoviruses	Associated with occasional outbreaks of respiratory and enteric disease
Porcine adenoviruses	Usually subclinical infections, occasionally cause diarrhoea
Fowl adenoviruses	Frequently isolated from healthy birds or following respiratory disease. Associated with quail bronchitis and inclusion body hepatitis

which may occur within weeks of clinical recovery in about 20% of affected animals, usually resolves spontaneously. Recovered animals have life-long immunity.

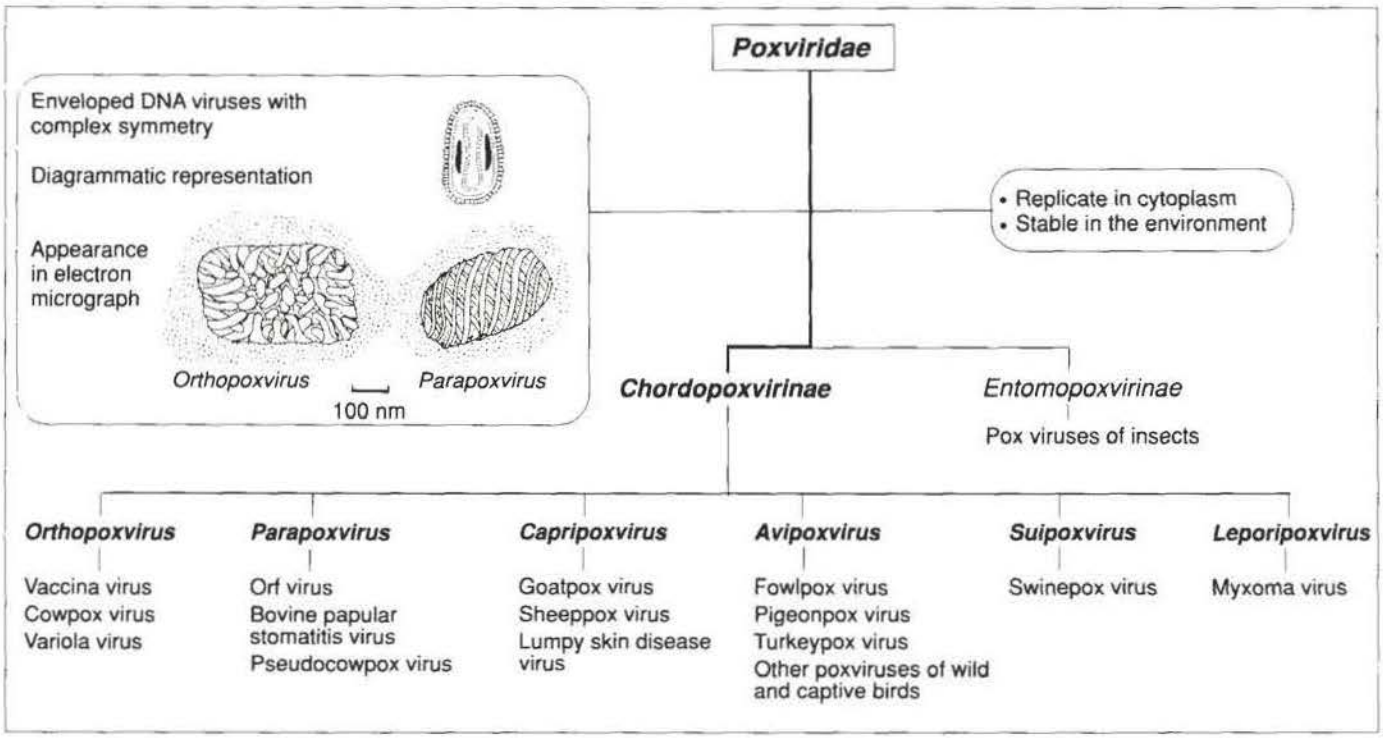
A history of fever, sudden collapse and abdominal pain in young, unvaccinated dogs may suggest infectious canine hepatitis. In dogs that have died, the demonstration of basophilic intranuclear inclusion bodies in hepatocytes, Kupffer cells and endothelial cells is confirmatory. Viral antigen can be demonstrated by immunofluorescence in cryostat sections of liver. A PCR method for detecting viral DNA in clinical specimens has been described.

Inactivated and modified live vaccines are available. Occasionally, vaccination with modified live CAV-1 vaccines results in mild nephropathy with shedding of virus in urine and, in some instances, corneal opacity. These side effects do not occur with modified live CAV-2 vaccines which stimulate effective long-lasting immunity to CAV-1. Inactivated CAV-1 vaccines, which do not induce obvious side-effects, require boosters at more frequent intervals in order to maintain adequate antibody levels.

Infection with canine adenovirus type 2

Canine adenovirus type 2, which is readily transmitted by aerosols, replicates in both the upper and lower respiratory tract. Clinical signs are generally mild or inapparent. Affected dogs may present with clinical signs similar to those of canine infectious tracheobronchitis (kennel cough). Most dogs recover and are immune to subsequent challenge. Occasional cases of bronchopneumonia may develop due to secondary bacterial infection. Virus shedding continues for about 9 days after infection.

50 *Poxviridae*



The family *Poxviridae* contains the largest viruses which cause disease in domestic animals. The family is divided into two subfamilies, *Chordopoxvirinae*, the poxviruses of vertebrates, and *Entomopoxvirinae*, the poxviruses of insects. Genetic recombination within genera results in extensive serological cross-reactions and cross-protection. Replication in the cytoplasm of host cells takes place within defined areas ('viral factories'). Virus envelopes are derived from host cell membranes. Both enveloped and non-enveloped forms of the virus are infectious. Virions are stable at room temperature under dry conditions but sensitive to heat, detergents, formaldehyde and oxidizing agents. The genera differ in ether sensitivity.

Infections with poxviruses usually result in vesicular skin lesions (Table 50.1). Smallpox, caused by variola virus, was formerly a human disease of major international significance. The use of vaccinia virus for the prevention of smallpox, first introduced by Jenner in the late 18th century, eventually led to the eradication of this highly contagious disease at the close of the twentieth century.

Clinical infections

Transmission of poxviruses can occur by aerosols, by direct contact, by mechanical transmission through arthropods and through fomites. Skin lesions are the principal feature of these infections. Several virus-encoded proteins are released from infected cells, including a homologue of epidermal growth factor which stimulates cell proliferation. Typically, pox lesions begin as macules and progress through papules, vesicles and pustules to scabs which detach, leaving a scar. In generalized infections there is a cell-associated viraemia and recovered animals have solid immunity. Some localized pox infections may induce transient immunity and reinfection can occur.

Three closely related parapoxviruses, namely pseudocowpox virus, bovine papular stomatitis virus and orf virus, infect ruminants. These viruses are transmissible to humans, producing lesions which are clinically similar. Moreover, the three viruses are morphologically indistinguishable and identification of the causal agent relies on nucleic acid analysis.

Capripoxviruses are economically important viruses producing generalized infections with significant mortality in domestic ruminants. Sheeppox virus, goatpox virus and lumpy skin disease virus are closely related and share a group-specific structural protein (p32), which allows the same vaccine to be used against each virus.

Many avian species are susceptible to infection with members of the genus *Avipoxvirus*. Although antigenic relationships exist among avian poxviruses, this

Table 50.1 Members of the *Poxviridae* of veterinary significance.

Virus	Genus	Host species	Significance of infection
Vaccinia virus	*Orthopoxvirus*	Wide host range	Infections in sheep, water buffaloes, rabbits, cattle, horses and humans. Used as a recombinant virus vector for rabies vaccine
Cowpox virus	*Orthopoxvirus*	Rodents, cats, cattle	Species of small rodents are the likely reservoir hosts. Cats are the principal incidental hosts; infection results in skin lesions. Rare cause of teat lesions in cattle. Transmissible to humans
Uasin gishu virus	*Orthopoxvirus*	Unknown wildlife reservoir, horses	Rare disease, reported in Kenya and neighbouring African countries. Causes papilloma-like skin lesions in horses
Camelpox virus	*Orthopoxvirus*	Camel	Widely distributed in Asia and Africa. Causes systemic infection with typical pox lesions; severe infection in young camels
Pseudocowpox virus	*Parapoxvirus*	Cattle	Common cause of teat lesions in milking cows; causes milker's nodule in humans
Bovine papular stomatitis virus	*Parapoxvirus*	Cattle	Produces mild papular lesions on the muzzle and in the oral cavity of young cattle. Transmissible to humans
Orf virus	*Parapoxvirus*	Sheep, goats	Primarily affects young lambs; causes proliferative lesions on the muzzle and lips. Transmissible to humans
Sheeppox / Goatpox virus	*Capripoxvirus*	Sheep, goats	Endemic in Africa, Middle East and India. Causes generalized infection with characteristic skin lesions and variable mortality
Lumpy skin disease virus	*Capripoxvirus*	Cattle	Endemic in Africa. Causes generalized infection with severe lesions and variable mortality
Swinepox virus	*Suipoxvirus*	Pigs	Causes mild, skin disease. Occurs worldwide. Transmitted by the pig louse (*Haematopinus suis*)
Fowlpox virus	*Avipoxvirus*	Chickens, turkeys	Causes lesions on the head and on the oral mucous membrane. Occurs worldwide. Transmitted by biting arthropods
Myxoma virus	*Leporipoxvirus*	Rabbits	Causes mild disease in cottontail rabbits, the natural host, and severe disease in European rabbits (myxomatosis). Introduced into Europe, Australia and Chile as a biological control measure

relatedness is variable. Virus species within the genus, named in accordance with their affinity for particular host species, include fowlpox virus, canarypox virus, pigeonpox virus and turkeypox virus. The type species of the genus is fowlpox virus.

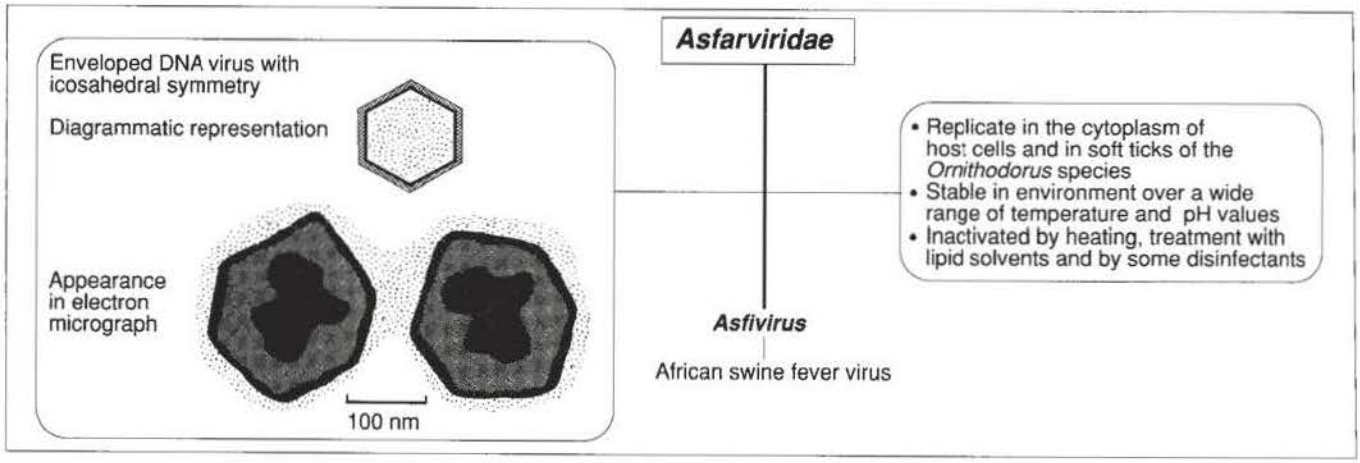
Diagnosis

Diagnosis can often be made solely on clinical grounds. Skin biopsies or postmortem specimens may be used for laboratory confirmation. Eosinophilic intracytoplasmic inclusions may be demonstrable histologically in epidermal cells. Electron microscopy can be used for the rapid identification of poxvirus particles in material from lesions. Parapoxviruses can be readily distinguished from members of the other genera. For some species, virus may be isolated in testis or kidney cell monolayers. An antigen-trapping ELISA has been developed for the detection of capripoxvirus antigen.

Control

Modified live and inactivated vaccines are available for a number of poxviruses and control is based on annual vaccination. Inactivated vaccines are less effective than modified live vaccines because cell-mediated immunity is the predominant protective response. A recombinant vaccine providing protection against lumpy skin disease and rinderpest has been developed. In flocks endemically infected with orf virus, control is based on the use of a fully virulent live vaccine derived from scab material or cell culture. Ewes should be vaccinated by scarification in the axilla at least eight weeks before lambing. When close to lambing, they must be moved to a new grazing area in order to minimize exposure of lambs to infectious vaccinal scab material.

51 *Asfarviridae* and *Bornaviridae*



Asfarviridae

African swine fever virus (ASFV), formerly assigned to the family *Iridoviridae*, has recently been reassigned to a newly created family, *Asfarviridae*, containing the single genus *Asfivirus*. African swine fever virus is the type species of this genus. Virions are 175 to 215 nm in diameter and consist of a membrane-bound nucleoprotein core inside an icosahedral capsid, surrounded by an outer lipid-containing envelope. This complex virus contains more than 50 proteins, including a large number of structural proteins and several virus-encoded enzymes required for transcription and post-translational modification of mRNA. The genome consists of a single molecule of linear double-stranded DNA. Following replication in the cytoplasm of host cells, virus is released either by budding through the plasma membrane or following cellular disintegration. African swine fever virus is stable in the environment over a wide range of temperature (4°C to 20°C) and pH values. The virus may persist for months in meat. Infectivity can be destroyed by heating and by treating with lipid solvents and some disinfectants such as ortho-phenylphenol.

African swine fever

African swine fever (ASF) is an economically important viral disease of pigs, characterized by fever, haemorrhages in many tissues and a high mortality rate. It is endemic in sub-Saharan Africa and Sardinia. Domestic and wild pigs are the only species susceptible to infection. In Africa, ASFV is maintained in a sylvatic cycle involving soft ticks of the genus *Ornithodorus* and inapparent infection of warthogs and bushpigs. Adult warthogs with persistent inapparent infection rarely develop viraemia. In contrast, young warthogs develop viraemia and are a major source of virus for soft ticks. Replication of virus occurs in the ticks and both trans-ovarial and trans-stadial transmission have been described. Soft ticks feed for short periods on hosts before dropping off and sheltering in crevices in walls or cracks in the ground. The presence of ticks in a particular region makes the eradication of ASF difficult. Virulent strains of ASFV, producing high mortality in infected animals, are widely distributed in Africa. Many isolates from other parts of the world are less virulent and mortality rates are usually below 50%.

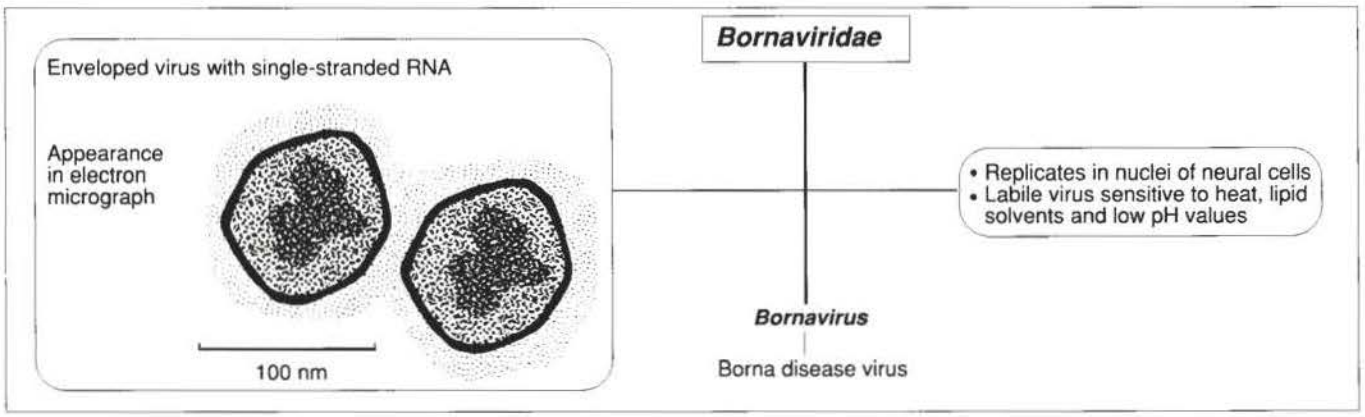
Following infection of domestic pigs with virulent virus, body fluids and tissues contain large quantities of virus until death or recovery occurs. Ingestion of uncooked meat from infected warthogs or domestic pigs is a major method of transmission. Spread can also occur by direct contact, usually through oral or nasal secretions. Occasionally, animals become infected by contact with blood shed as a result of fighting. Indirect transmission can occur through contaminated transport vehicles, fomites and footwear. Feeding uncooked swill is an important mechanism of spread of ASF internationally, with outbreaks often starting in herds close to airports and harbours. Pigs which have recovered from clinical disease may remain infected for long periods. Carrier pigs are considered to be important sources of virus dissemination.

Infection in domestic pigs is usually acquired via the oronasal route. The virus replicates primarily in cells of the lymphoreticular system; it can also infect megakaryocytes, endothelial cells, renal epithelial cells and hepatocytes. The clinical signs of ASF, which range from inapparent to peracute, relate to the challenge dose and virulence of the virus and to the route of infection. The incubation period is typically five to seven days in acute cases. Animals with peracute disease die suddenly without premonitory clinical signs. Fever, inappetence, depression and recumbency are features of acute disease. Cutaneous hyperaemia and, in some cases, haemorrhages may be evident. Other signs include dyspnoea, conjunctivitis, diarrhoea, bleeding from the nose and rectum, and abortion. The mortality rate is high. Subacute disease has a course of three to four weeks. Clinical signs include pneumonia, swollen joints, emaciation, depression and inappetence. Mortality rates,

which are variable, depend on the age and general health of infected pigs. Animals may recover and appear clinically normal or may develop a chronic form of the disease, which usually occurs in regions where ASFV is endemic.

Laboratory confirmation of ASF is based on detection of ASFV using tests such as direct immunofluorescence and haemadsorption. The polymerase chain reaction can be used to detect DNA from ASFV in tissues unsuitable for virus isolation or antigen detection. Antibodies persist for long periods in recovered animals and serological testing may be the only means of detecting animals infected with strains of low virulence.

Restriction of pig movement, serological monitoring of carrier pigs, and prevention of contact between domestic pigs and warthogs or ticks are important control measures in countries where the disease is endemic. Eradication of tick species which act as vectors of ASFV is an essential part of a control programme. A successful vaccine is not yet available. Countries maintain disease-free status by prohibiting importation of pigs and pig products. Waste food scraps from aircraft and ships must be boiled before inclusion in pig feed. In the face of an outbreak of ASF in countries free of infection, an eradication policy is implemented. The occurrence of low virulence strains renders eradication difficult.



Bornaviridae

The family *Bornaviridae* contains a single genus *Bornavirus*. The sole member of the genus is Borna disease virus (BDV). This enveloped virus, which has only recently been demonstrated by electron microscopy, is spherical, with a diameter of about 90 nm. The envelope surrounds an inner core, 50 to 60 nm in diameter. The genome consists of a single molecule of negative-sense, single-stranded RNA. Replication occurs in the nucleus of host cells with budding at the cell surface. This labile virus is sensitive to heat, lipid solvents and low pH values.

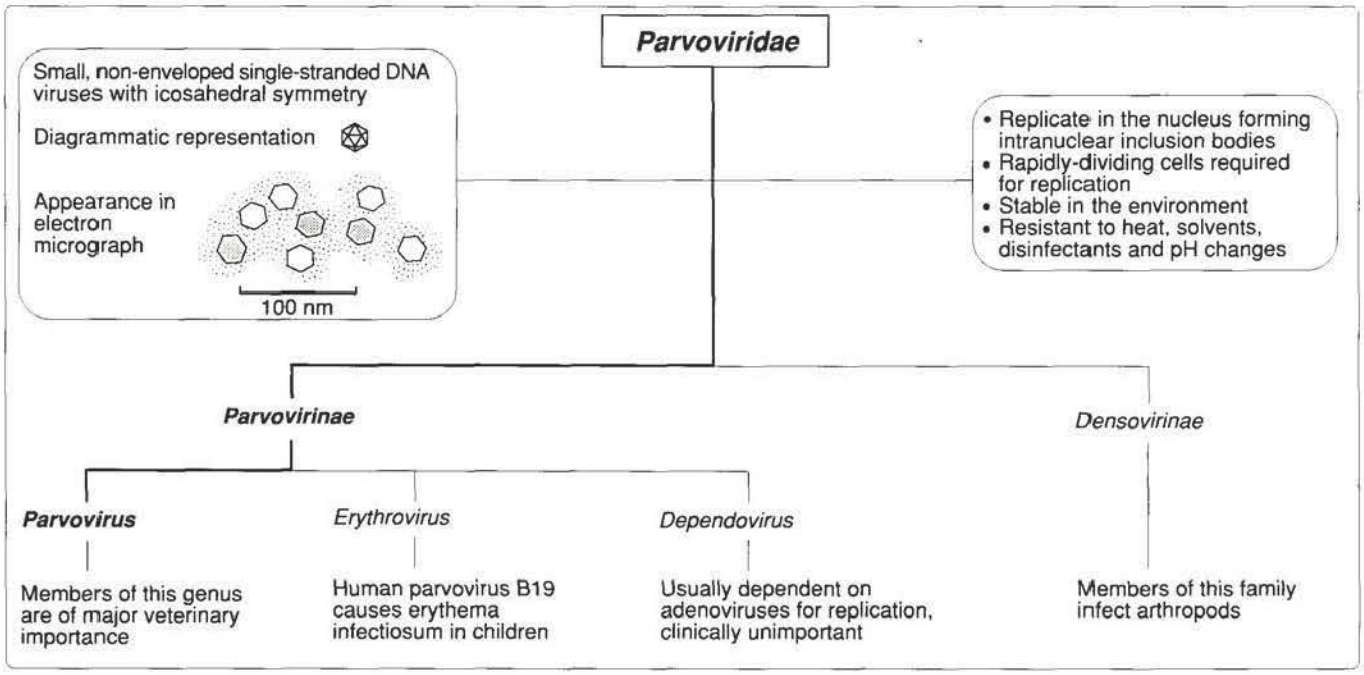
Borna disease

First described over 200 years ago, this fatal neurological disease of horses is named after Borna, the town in Saxony where it was first recorded. The disease occurs sporadically in Germany, Switzerland and other parts of Europe. Sero-epidemiological studies, however, indicate a wide geographical distribution. Neurological disease attributed to BDV has been described in horses and cats. Serological evidence of infection has been recorded in other species including sheep, rabbits and ostriches. It is thought that virus may be transmitted through ingestion or inhalation. Most cases of Borna disease occur in spring and early summer; prevalence varies from year to year. It has been suggested that rodents may act as reservoir hosts. Persistent infections can be established experimentally in rats.

Following oronasal infection, the virus gains entry to the CNS by intra-axonal spread, either through the olfactory nerve or through nerves supplying the oropharyngeal and intestinal regions. Borna disease has been described mainly in young horses. The incubation period, which is highly variable, ranges from weeks to several months. Factors which may influence the severity of clinical signs include the age and immunological status of the infected animal and the strain of infecting virus. On farms where infection in horses is present, clinical disease is usually confined to individual animals. Clinical signs include fever, somnolence and evidence of neurological disturbance. Ataxia, pharyngeal paralysis and hyperaesthesia may be present. The course of the disease is up to three weeks and mortality rates may reach 100%. Surviving horses have permanent CNS damage and may exhibit recurrent episodes of neurological disturbance. 'Staggering disease' in cats has been associated with BDV infection. In defined areas, some sheep flocks have large numbers of seropositive animals.

Borna disease may vaguely resemble other neurological conditions in the horse. However, the distribution of lesions in the CNS differs from that in other equine encephalomyelitides and, if eosinophilic intranuclear inclusions (Joest-Degen bodies) are present, they may be confirmatory. Viral antigen can be demonstrated in the brain by immunohistochemical methods. Demonstration of antibodies in serum or in cerebrospinal fluid by immunofluorescence or ELISA may aid diagnosis. Reverse transcriptase-PCR for the demonstration of BDV-RNA is a valuable diagnostic tool. Control is difficult due to the sporadic nature of the disease. Although BDV does not appear to be readily transmitted by infected horses, seropositive animals should be isolated. Standard hygienic measures should be applied to suspect animals.

52 *Parvoviridae*



Viruses belonging to the family *Parvoviridae* (Latin *parvus*, small) range in size from 18 to 26 nm in diameter and possess a linear genome of single-stranded DNA. Parvoviruses replicate only in the nuclei of dividing host cells, a feature which determines the tissues targeted. After entering a cell, the virion is uncoated and its single-stranded DNA genome is converted to double-stranded DNA by DNA polymerases in the nucleus. Following viral replication, cell lysis occurs as virions are released. Many parvoviruses of vertebrates agglutinate erythrocytes and haemagglutination inhibition by specific antisera is widely used for their identification.

Clinical infections

Parvoviruses can infect many domestic and wild animals (Table 52.1). Mink enteritis virus, canine parvovirus and racoon parvovirus are considered to be host-range mutants of feline panleukopenia virus. Although most members of the group produce acute systemic diseases, some such as canine minute virus and bovine parvovirus, are of uncertain pathogenic significance. The most important parvoviral diseases of domestic animals are feline panleukopenia, canine parvovirus infection and porcine parvovirus infection.

Feline panleukopenia

Feline panleukopenia, also known as feline infectious enteritis or feline distemper, is a highly contagious generalized disease of domestic and wild cats. The disease, which is worldwide in distribution, is one of the most common feline viral infections.

Infection is generally endemic in unvaccinated cat populations with disease occurring predominantly in young recently-weaned kittens as maternally-derived antibody levels wane. The disease may have a cyclical or seasonal pattern which is related to the births of kittens. Transplacental infection occurs in fully susceptible queens with effects on the foetus ranging from cerebellar hypoplasia to foetal death. High rates of virus excretion occur during the acute stage of the disease, mainly in faeces. In cool, moist, dark environments, infectivity may last for more than a year.

Following ingestion or inhalation, replication occurs in the oropharynx and associated lymph nodes. Viraemia develops within 24 hours, producing infection of mitotically active cells in other tissues, particularly the cells of the intestinal crypts and the lymphopoietic cells of the bone marrow, thymus, lymph nodes and spleen. Destruction of these target tissues results in panleukopenia and villous atrophy. Disease is characterized by sudden onset of pronounced depression, anorexia and fever. Vomiting, sometimes accompanied by diarrhoea or dysentery, follows. The mortality rate ranges from 25% to 90%.

Diagnosis is usually based on demonstration of virus particles by electron microscopy or detection of viral antigen using ELISA or haemagglutination in faecal samples from cats with acute disease. Typical histopathological changes may be present in sections of the ileum and jejunum.

Vaccination is the principal control measure. There is only one serotype of feline panleukopenia virus and immunity following natural infection is strong and long-lasting. As

Table 52.1 Parvoviruses of veterinary significance.

Virus	Hosts	Consequences of infection
Feline panleukopenia virus	Domestic and wild cats	Highly contagious systemic and enteric disease most common in weaned kittens, manifested as depression, vomiting, diarrhoea. Intrauterine infection: abortion or cerebellar ataxia in neonatal kittens
Canine parvovirus (Canine parvovirus 2)	Dogs	Highly contagious enteric disease with depression, vomiting, dysentery and immunosuppression. Intrauterine or perinatal infection: myocarditis in pups (now rare)
Porcine parvovirus	Pigs	Major cause of stillbirths, mummified foetuses, embryonic deaths and infertility (SMEDI syndrome)
Mink enteritis virus	Mink	Generalized disease of mink kits, analagous to feline panleukopenia
Aleutian mink disease virus	Mink, ferrets	Chronic, progressive disease of mink homozygous for pale coat colour. Persistent viraemia, plasmacytosis, hypergammaglobulinaemia and immune complex-related lesions
Goose parvovirus (goose plague virus)	Geese	Highly contagious, fatal disease of 8-30 day old goslings (Derzsy's disease): hepatitis, myositis, including myocarditis
Canine minute virus (Canine parvovirus 1)	Dogs	Role of virus in disease is uncertain; serological surveys suggest the virus is widespread
Bovine parvovirus	Cattle	Associated with sporadic outbreaks of diarrhoea in calves

clinical infections cause heavy environmental contamination, premises should be thoroughly disinfected.

Canine parvovirus infection

Infection with canine parvovirus emerged in the late 1970s as a worldwide disease in dogs, with high morbidity and mortality. Acute or subacute heart failure in pups infected *in utero* or during the perinatal period, was a common manifestation of the disease. With the gradual development of immunity in the adult dog population, the clinical pattern of the disease changed. The most common clinical presentation now encountered is acute enteric disease in young dogs between weaning and 6 months of age. Canine parvovirus is considered to be a host-range mutant of feline panleukopenia virus or a closely related parvovirus. Many canine species are susceptible to infection and transmission is predominantly by the faecal-oral route.

The virus replicates initially in pharyngeal lymphoid tissues and Peyer's patches. Viraemia develops and the main target tissues are those with rapidly multiplying cell populations. During the first two weeks of life there is active cardiac myocyte division allowing viral replication, with resultant necrosis and myocarditis. In older pups, the virus invades the actively dividing epithelial cells of the crypts in the small intestine. There may be extensive haemorrhage into the intestinal lumen in severely affected pups.

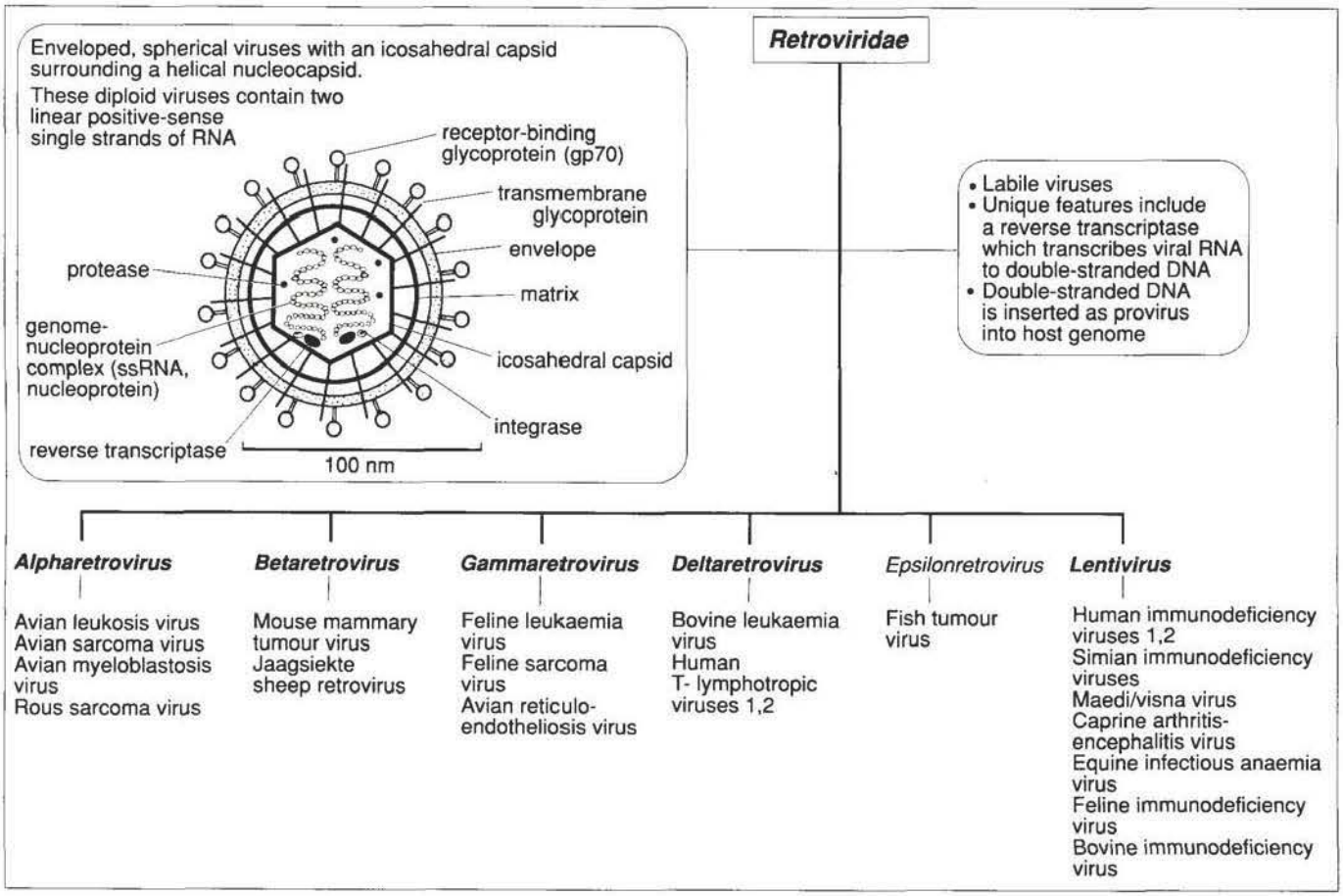
After a short incubation period of four to seven days, animals with enteric disease show sudden onset of vomiting and anorexia. Diarrhoea, often blood-stained, develops within 48 hours. Affected dogs deteriorate rapidly due to dehydration and weight loss. Definitive diagnosis early in the course of the disease relies on the demonstration of virus or viral antigen in faeces. In fatal cases, the nature and distribution of the gross and microscopic enteric lesions may point to a parvoviral infection.

As vaccination alone usually cannot be relied on to control the cycle of endemic parvovirus infection in kennels, thorough disinfection of premises must be carried out following a disease outbreak.

Porcine parvovirus infection

Porcine parvovirus is an important cause of reproductive failure in pigs worldwide. On farms where the disease is endemic, many sows are immune. Maternally-derived immunity usually persists for about four months, but it can persist in some pigs until they are six to nine months of age. During this period, the maternally-derived antibodies may interfere with the development of active immunity and consequently some gilts can be seronegative and susceptible to infection at mating. The virus has a predilection for the mitotically active cells of foetal tissues. Transplacental infection in pregnant sows occurs 10 to 14 days after exposure to the virus. The major damage to foetuses arises before onset of immunocompetence, at about 60 to 70 days of gestation. Infection of embryos in the first weeks of life results in death and resorption. When infection occurs later in gestation, but before day 70, foetuses die and become mummified. Infection after 70 days of gestation usually results in the birth of healthy seropositive piglets. Demonstration of viral antigen in cryostat sections of foetal tissues by immunofluorescence is reliable and sensitive. Control is based on exposure of gilts and susceptible sows to porcine parvovirus prior to mating. This can be achieved by vaccination or by exposing animals to contaminated faeces or to placental or foetal tissue from infected sows.

53 *Retroviridae* 1



Retroviruses (Latin *retro*, backwards) are labile, enveloped RNA viruses, 80 to 100 nm in diameter. The family name refers to the presence, in the virion, of a reverse transcriptase which is encoded in the viral genome. Reverse transcriptase acts as an RNA-dependent DNA polymerase, which transcribes from RNA to DNA. Under the influence of the reverse transcriptase, double-stranded DNA copies of the viral genome are synthesized in the cytoplasm of the host cell. During this process, repeat base sequences, containing several hundred base-pairs and called long terminal repeats (LTR), are added to the ends of the DNA transcripts. Transcripts are integrated into the chromosomal DNA, as provirus, at random sites through the action of viral integrase. The sites of proviral integration determine the extent and nature of cellular changes. The LTR contain important promoter and enhancer sequences.

A high mutation rate is a feature of retroviral replication because errors are relatively frequent during reverse transcription. In addition, recombination between retroviral genomes in doubly-infected cells can occur. Consequently, antigenically different retroviruses frequently emerge and classification of species and subtypes often proves difficult.

Retroviruses can be categorized as endogenous or exogenous. Endogenous retroviruses occur widely among vertebrates. These viruses are consistently present in germline cells and are transmitted only as provirus in germ cell DNA from parent to offspring. They are regulated by cellular genes and are usually silent. Exogenous retroviruses are capable of horizontal transmission between members of the host species.

Retroviruses are sensitive to heat, lipid solvents and detergents. Because of their diploid genomes, they are relatively resistant to UV light.

Lentiviruses

Lentiviruses (Latin *lentus*, slow) cause lifelong infections and are associated with diseases that have a long incubation period and an insidious protracted course. Lentiviruses of domestic animals are presented in Table 53.1.

Feline immunodeficiency virus infection

This condition of domestic cats was first reported in 1987 and is now recognised worldwide as an important cause of disease in cats. Five subtypes of FIV have been identified. Virus is shed mainly in the saliva and transmission usually occurs through

Table 53.1 Lentiviruses of domestic animals.

Virus	Hosts	Comments
Feline immuno-deficiency virus	Cats	Causes life-long infection with persistent viraemia and immunosuppression in cats over 5 years of age. Worldwide distribution
Equine infectious anaemia virus	Horses, mules, donkeys	Causes life-long infection with recurring febrile episodes. Anaemia is a prominent clinical sign
Maedi/visna virus	Sheep	Causes life-long infection with progressive respiratory disease (maedi) and indurative mastitis in older sheep. Clinical signs develop in a small percentage of infected animals. Infected sheep rarely develop progressive neurological disease (visna)
Caprine arthritis-encephalitis virus	Goats	Causes life-long infection. Associated with polyarthritis and indurative mastitis in adults and progressive nervous disease in kids. Common in dairy goat herds. Worldwide distribution
Bovine immuno-deficiency virus	Cattle	Widely distributed; pathogenicity currently uncertain

bites. Accordingly, infection rates are highest in free-roaming, adult male cats. Not all infected cats develop disease.

The virus replicates principally in $CD4^+$ (helper) T lymphocytes, producing a progressive deterioration in cell-mediated immunity due to depletion of these lymphocytes. The prevalence of clinical disease is highest in cats over six years of age. The course of the disease may be divided into an acute phase, a prolonged asymptomatic phase, a phase characterized by vague clinical signs and a terminal phase with marked immunodeficiency. Clinical signs are highly variable and include recurrent fever, leukopenia, anaemia, weight loss, lymphadenitis, chronic gingivitis and behavioural changes. Opportunistic infections are frequent in the terminal phase of the disease. Chronic stomatitis and gingivitis are common findings. Other manifestations include chronic respiratory, enteric and skin infections. Neurological signs, usually due to direct viral damage, develop in a small number of infected cats.

Diagnosis is primarily based on serological testing for antibodies. Commercial ELISA and immunoconcentration kit-sets are available. Alternative tests include immunoblotting and indirect immunofluorescence. Treatment is primarily aimed at the control of secondary infections. Control is based on prevention of exposure by separating infected and non-infected cats in multicat households, by preventing cats from roaming freely, by using seronegative queens for breeding and by screening all cats before introduction into seronegative populations. A commercial vaccine is not currently available.

Equine infectious anaemia

This disease affects horses, mules and donkeys in many countries. The virus is transmitted mechanically by haematophagous insects, particularly *Tabanus* species and *Stomoxys* species. Transmission occurs most often in the summer, during periods of high insect activity, in low-lying swampy areas close to woodlands. Iatrogenic transmission can occur through contaminated needles or surgical instruments.

The virus replicates in macrophages, monocytes and Kupffer cells. Infected horses fail to eliminate the virus despite mounting a strong immune response. In the course of viral replication, mutations frequently arise and can result in the emergence of new virus strains exhibiting antigenic variation in envelope glycoproteins (antigenic drift). Febrile episodes and marked immune stimulation signal the emergence of these new strains. Non-neutralizing antibodies produced against virus early in the course of infection lead to the formation of immune complexes. Such immune complexes activate complement, contributing to fever, anaemia and thrombocytopenia, and initiating glomerulonephritis. Haemolysis, enhanced erythrophagocytosis and depressed erythropoiesis are responsible for the anaemia in chronically affected horses. In most animals, clinical episodes eventually cease, probably as a consequence of a broad-based neutralizing response against a wide range of viral epitopes.

Laboratory confirmation of infection is based on the demonstration of serum antibodies to the core virus protein p26. The serological test recognized for international trade is the AGID test (Coggins test). Restriction of animal movement accompanied by detection and removal of seropositive animals are used to minimize the risk of disease spread.

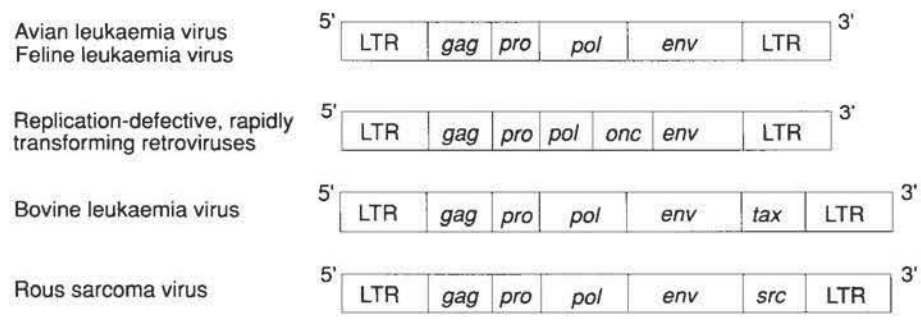
Small ruminant lentivirus group

Two distinct lentiviruses have been described in small ruminants, maedi/visna virus (MVV) and caprine arthritis-encephalitis virus (CAEV). These viruses are closely related and cause persistent infections and comparable disease syndromes. Each virus can infect both species. Genomic analyses of these ovine and caprine lentivirus isolates suggest that they evolved from a common ancestral genotype. The current view is that they comprize a heterogeneous group with a variable host range and different pathogenic capabilities.

Infection is frequently subclinical. The clinical severity of disease is influenced by viral virulence, the age of the host when exposed and other host factors. Virus production occurs in infected monocytes following their development into macrophages. The immune response is not fully effective and probably contributes to the pathogenesis of the disease.

Laboratory confirmation relies on detection of virus-specific antibodies. The most commonly used assays are AGID and ELISA. Control is based on test and segregation programmes. The milk from infected animals is an important source of infection and newborn animals should be reared separately from their infected dams.

Schematic representation of the important genes present in oncogenic viruses



env gene encoding envelope glycoproteins
gag gene encoding group-specific antigen (core and capsid proteins)
LTR long terminal repeat sequences
onc oncogene
pol gene encoding polymerase
pro gene encoding protease
src sarcoma gene
tax transactivating gene

Retroviruses in the genera *Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus* and *Deltaretrovirus* are frequently referred to as oncogenic retroviruses because they can induce neoplastic transformation in cells which they infect (Table 54.1).

On the basis of the interval between exposure to the virus and tumour development, exogenous oncogenic retroviruses are designated either as slowly transforming (*cis*-activating) viruses or as rapidly transforming (transducing) viruses. Slowly transforming retroviruses induce B cell, T cell or myeloid tumours after long incubation periods. For malignant transformation to occur, the provirus must be integrated into the host cell DNA close to a cellular oncogene (c-*onc*, proto-oncogene), resulting in interference with the regulation of cell division (insertional mutagenesis). Multiple insertions of the provirus into the host cell genome results in exaggerated gene expression and over-production of a transformation-associated protein. Rapidly transforming retroviruses, which can induce tumour formation after short incubation periods, contain viral oncogenes (v-*onc*). More than a dozen different oncogenes have been identified in transforming avian retroviruses. Viral oncogenes are considered to be cellular oncogenes acquired by recombination during virus evolution. If the oncogene is integrated into the viral genome without loss of replicative virus genes, as in Rous sarcoma virus, the retrovirus is described as replication-competent. Frequently, as a consequence of cellular oncogene integration, existing viral sequences necessary for replication are deleted. Such replication-defective retroviruses, which cannot multiply without helper viruses, are rarely transmitted under normal field conditions. The protein products of oncogenes may act as hormone or growth factor receptors, transcription control factors and kinases in signal transduction pathways. A third method of tumour induction is exemplified by bovine leukaemia virus which depends on the *tax* gene encoding a protein capable of up-regulating both viral LTR and cellular promoter sequences, even when the provirus is integrated into a different chromosome (*trans*-activation).

Feline leukaemia and associated clinical conditions

Infection with feline leukaemia virus (FeLV) not only results in feline leukaemia but is also associated with a variety of other clinical conditions. Isolates of FeLV are assigned to three subgroups (A, B and C) on the basis of differences in the gp70 envelope glycoprotein. Feline leukaemia virus A (FeLV-A), the predominant subgroup, is isolated from all FeLV-infected cats. Viruses of subgroup B, which arise through recombination between the *env* genes of FeLV-A and endogenous FeLV-related proviral DNA, are present in about 50% of isolates. Cats that are infected with both FeLV-A and FeLV-B have a higher risk of developing tumours than those infected with FeLV-A alone. Each FeLV-C isolate is unique, arising *de novo* in a FeLV-A infected cat through mutations in the receptor-binding region of the FeLV-A *env* gene. A combination of subgroups A and C or A, B and C viruses is found in 1% of persistently viraemic cats. Once generated, FeLV-C viruses rapidly cause a fatal anaemia and consequently are not transmitted to other cats.

Close contact is required for transmission of this labile virus and the incidence of infection is related to population density. Highest infection rates are found in catteries and multicat households. Large amounts of virus are shed in saliva. Infection is usually acquired by licking, grooming and through bite wounds. Young kittens are more susceptible to infection than adults. Although maternally-derived antibody is protective in kittens up to six weeks of age, a significant proportion of those exposed before 14 weeks of age become persistently infected. Such animals constitute the main reservoir of FeLV and are prone to develop an FeLV-related disease. Because the production of virus particles requires cellular DNA synthesis,

Table 54.1 Oncogenic retroviruses of veterinary importance.

Genus	Virus	Hosts	Comments
Alpharetrovirus	Avian leukosis virus	Chickens, pheasants, partridge, quail	Endemic in commercial flocks. Exogenous and endogenous transmission of virus can occur. Causes lymphoid leukosis in birds between 5 and 9 months of age
Betaretrovirus	Jaagsiekte sheep retrovirus	Sheep	Causes jaagsiekte, a slowly progressive neoplastic lung disease of adult sheep which is invariably fatal. Occurs worldwide except in Australasia
	Enzootic nasal tumour virus	Sheep	Closely related to jaagsiekte sheep retrovirus. Causes adenocarcinoma of low grade malignancy, which affects the nares
Gammaretrovirus	Feline leukaemia virus	Cats	Important cause of chronic illness and death in young adult cats. Causes immunosuppression, enteritis, reproductive failure, anaemia and neoplasia. Worldwide in distribution
	Reticuloendotheliosis virus	Turkeys, ducks, chickens, quail, pheasants	Infection usually subclinical. Sporadic disease may present with anaemia, feathering defects, impaired growth or neoplasia. Disease outbreaks have occurred following use of vaccine contaminated with reticuloendotheliosis virus
Deltaretrovirus	Bovine leukaemia virus	Cattle	Causes enzootic bovine leukosis in adult cattle. A small percentage of infected cattle develop lymphosarcoma

tissues with high mitotic activity, such as bone marrow and epithelia, are targeted. Severe immunosuppression is caused by infection with certain strains of FeLV-A. This virus causes tumours, particularly lymphosarcoma, by several means, including insertional mutagenesis and recombination with a variety of cellular proto-oncogenes to produce rapidly transforming, replication-defective viruses. Examples of the latter are FeLVs isolated from thymic lymphomas, and feline sarcoma viruses (FeSV) that are isolated from rare multicentric fibrosarcomas in young cats. These viruses are not transmitted under natural conditions. The majority of persistently-infected cats die within three years of infection. About 80% of these cats die from non-neoplastic FeLV-associated disease. Anaemia, reduction in reproductive performance, enteritis and a variety of secondary infections are important features of the disease.

Detection of viral antigen in blood or saliva is the method commonly used for the laboratory diagnosis of feline leukaemia. Commercial ELISA and rapid immunomigration tests are available. A test and removal policy has been shown to be effective in eradicating infection from catteries. Several commercial vaccines are available. Vaccination does not provide complete protection and does not alter the course of infection in persistently-infected cats.

Enzootic bovine leukosis

This retroviral disease of adult cattle is characterized by persistent lymphocytosis and the development of B cell lymphosarcoma in a number of infected animals. The labile virus is intimately cell-associated and transmission usually takes place through transfer of blood or secretions such as milk containing infected lymphocytes. Less than 10% of calves born to infected dams are infected at birth. Animals are usually infected between six months and three years of age. Iatrogenic transmission is important and has been linked to reuse of needles, multidose injectors, contaminated surgical instruments and rectal examination procedures. The primary target cell is the B lymphocyte. Although infections are life-long, most animals remain subclinically infected. About 30% of infected animals develop persistent lymphocytosis without clinical signs of disease. A small percentage eventually develop lymphosarcoma as adults. The presenting signs relate to the sites of tumour formation.

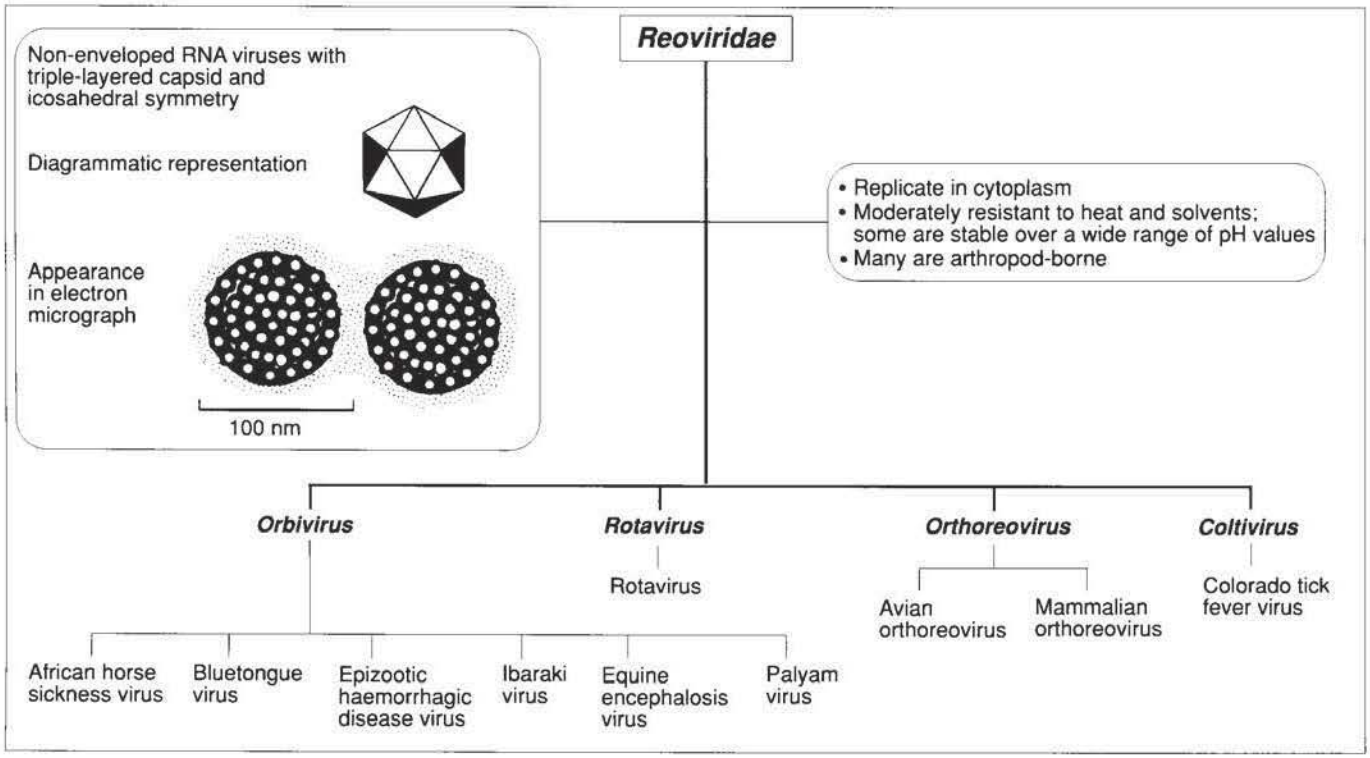
Several serological tests including AGID, ELISA and radioimmune assay are suitable for the detection of antibodies to bovine leukaemia virus. No commercial vaccine is currently available. Test and removal strategies are used in eradication programmes.

Jaagsiekte

This lentiviral disease, also called ovine pulmonary adenomatosis, is a slowly-progressing neoplastic disease of adult sheep. Respiratory exudates from affected sheep are infectious and transmission occurs by the respiratory route. Close contact facilitates spread of infection with the incidence of disease highest in housed animals. Within an infected flock, disease incidence may be up to 20%.

The virus replicates in two types of pulmonary cells, type II alveolar cells and non-ciliated bronchial cells. Tumours arising from these cell types progressively replace normal lung tissue leading to death from asphyxia. The incubation period may range from several months up to two years. Affected animals are usually three to four years of age, in poor bodily condition, and display respiratory embarrassment. Secondary pasteurellosis is a frequent complication. A clinical diagnosis is usually confirmed by histopathological examination. The incidence of disease in a flock can be reduced by strict isolation and elimination of suspect animals immediately after clinical or laboratory confirmation.

55 *Reoviridae*



Viruses in the family *Reoviridae* were originally isolated from respiratory and enteric sources without any associated disease, namely orphan. These icosahedral viruses, 60 to 80 nm in diameter, are non-enveloped and possess a layered capsid which is composed of concentric protein shells. The genome of the virion is composed of ten to twelve segments of double-stranded RNA. Genetic reassortment readily takes place in cells co-infected with viruses of the same species. Replication occurs in the cytoplasm of host cells, often with the formation of intracytoplasmic inclusions. The family contains nine genera. Members of the genera *Orthoreovirus*, *Rotavirus* and *Orbivirus* infect animals and humans. Members of the genus *Coltivirus*, which primarily infect rodents and humans, occasionally cause clinical disease in domestic animals. Other genera in the family contain viruses of plants, arthropods and fish. Viruses in the family are moderately resistant to heat, organic solvents and non-ionic detergents. Orthoreoviruses and rotaviruses are stable over a wide range of pH values unlike orbiviruses, which lose infectivity at low pH values.

Clinical infections

Reoviruses, which are widespread in nature, have been isolated from many animal species (Table 55.1). Mammalian and avian orthoreoviruses possess distinct group antigens. Avian orthoreoviruses have been implicated in arthritis, tenosynovitis, chronic respiratory disease and enteritis. Rotaviruses cause acute diarrhoea in young intensively-reared farm animals. Transmission of orthoreoviruses and rotaviruses occurs through contact with contaminated faeces.

Within the 19 currently recognized serogroups (species) of orbiviruses, there are defined serotypes and, in addition, antigenic complexes. The main serogroup-specific antigen is the immunodominant outer core protein VP7. Individual serotypes are distinguished by serum neutralization assays utilizing antibodies against outer capsid proteins. African horse sickness and bluetongue are particularly important diseases caused by orbiviruses. Epizootic haemorrhagic disease of deer and Ibaraki disease in cattle, both caused by closely-related orbiviruses, have clinical effects in these species similar to those of bluetongue in sheep. Infection with equine encephalosis virus has been recognized only in South Africa. Serological evidence suggests that this infection is widespread but acute disease occurs only sporadically. African horse sickness, bluetongue and epizootic haemorrhagic disease of deer are transmitted by arthropods, especially by *Culicoides* species.

Enteric disease caused by rotaviruses in young animals

Rotaviruses cause diarrhoea in intensively-reared young farm animals worldwide. Isolates are divided into several antigenically-distinct serogroups (A to F), also termed species, based on reactions with the major capsid protein, VP6. Most isolates belong to serogroup A. High titres of virus (10^9 virus particles per gram of faeces) are excreted by clinically affected animals. Because the virus is stable in the environment,

Table 55.1 Viruses of veterinary importance in the family *Reoviridae*.

Genus	Virus	Comments
Orbivirus	African horse sickness virus	Arthropod-borne infection of *Equidae*, principal vector *Culicoides* species. Endemic in Africa. High mortality rate
	Bluetongue virus	Arthropod-borne infection of sheep, cattle, goats and wild ruminants. Principal vector *Culicoides* species. Severe disease in some species of deer. Teratogenic effects. Clinical disease rare in cattle
	Epizootic haemorrhagic disease virus	Arthropod-borne infection of deer, cattle and buffalo. Principal vector *Culicoides* species. Clinically similar to bluetongue. Important disease of deer in North America. Subclinical infection occurs in cattle. Eight serotypes recognized
	Ibaraki virus	Member of the epizootic haemorrhagic disease virus serogroup. Acute febrile disease of cattle similar to bluetongue. Probably arthropod-borne. Present in south-east Asia
	Equine encephalosis virus	Reported in South Africa. Majority of infections subclinical. Sporadic cases of acute fatal disease. Cerebral oedema, fatty liver and enteritis are prominent features
	Palyam virus	Arthropod-borne disease of cattle. Causes abortion and teratogenic effects. Recorded in southern Africa, south-east Asia and Australia. Many viruses in the serogroup
Rotavirus	Rotaviruses	Occur in intensively-reared neonatal animals. Mild to severe diarrhoea, severity influenced by virulence of viral strain, age, colostral intake and management factors
Orthoreovirus	Avian orthoreoviruses	Important cause of viral arthritis/tenosynovitis in chickens. Multiple serotypes described. Turkeys and other avian species susceptible
	Mammalian orthoreoviruses	Associated with mild enteric and respiratory disease in many species, severity dependent on secondary infections. Three serotypes recognized
Coltivirus	Colorado tick fever virus	Rodent species act as reservoirs. Arthropod-borne, mainly ticks and also mosquitoes. Primarily of significance in humans; may cause encephalitis in children

premises may be heavily contaminated and intensively-reared animals are those most often affected. Diagnosis is based on electron microscopy or demonstration of viral antigen in faeces by ELISA and latex agglutination. Control involves measures aimed at reducing the levels of virus challenge in young animals while vaccination of pregnant dams can be used to enhance antibody levels in mammary secretions.

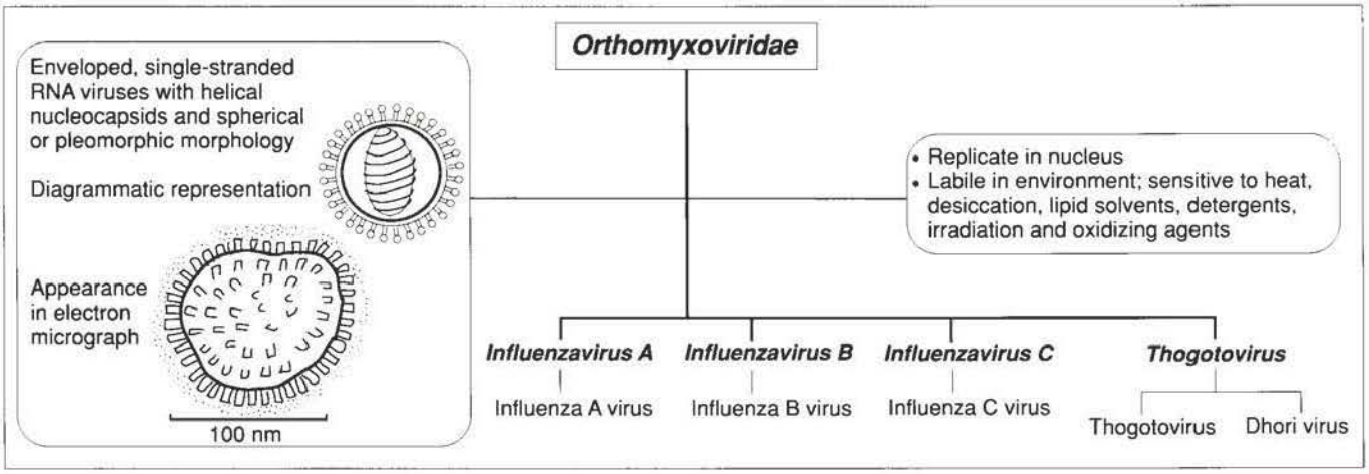
African horse sickness

This is a non-contagious List A disease of *Equidae* caused by African horse sickness virus (AHSV). Nine serotypes of this orbivirus constitute the African horse sickness serogroup. The disease is endemic in subtropical and tropical Africa. The virus is transmitted by haematophagous insects. Four forms of this febrile disease are recognized. A peracute pulmonary form is characterized by depression and nasal discharge, with rapid progression to severe respiratory distress. Mortality rates may approach 100%. A subacute cardiac form manifests as conjunctivitis, abdominal pain and progressive dyspnoea. Subcutaneous oedematous swellings of the head and neck are most obvious in the supraorbital fossae, palpebral conjunctiva and intermandibular space. In this form of the disease, the mortality rate is up to 70%. A third form of African horse sickness presents with both cardiac and pulmonary features. A mild or subclinical form, termed horse sickness fever, may be observed in zebras and donkeys. Vector control, quarantine of affected animals and vaccination are the main methods of control.

Bluetongue

This non-contagious List A viral disease of sheep and other domestic and wild ruminants is transmitted by biting insects. Twenty-four serotypes of bluetongue virus (BTV) have been described. Infection is of greatest significance in sheep and deer. In endemic areas, infection of cattle is common and usually inapparent. The viraemia in cattle commonly lasts several weeks, facilitating acquisition of virus by insect vectors. Consequently, cattle are considered to be important reservoirs of virus. The clinical presentation is highly variable, ranging from subclinical to severe disease with high mortality. Affected animals are febrile and depressed with vascular congestion of the lips and muzzle. Oedema of the lips, face, eyelids and ears develops. Erosions and ulcers are evident on the oral mucosa. Lameness may result from coronitis and laminitis. Mortality rate may be up to 30% and, in some outbreaks, may be higher. A presumptive diagnosis may be based on clinical findings and postmortem lesions. Confirmation requires isolation and identification of the virus or demonstration of BTV-specific antibodies. Live attenuated vaccines have been used successfully for many years and provide protection against virulent viruses of homologous serotype. Polyvalent vaccines are essential in regions where a number of serotypes are present. Attenuated vaccines may be teratogenic when used in ewes during the first half of gestation. Killed adjuvanted vaccines can induce protection but are more expensive to produce and require two inoculations.

56 *Orthomyxoviridae*



The family *Orthomyxoviridae* (Greek *orthos*, proper and *myxa*, mucus) contains those viruses which cause influenza in humans and animals. Orthomyxoviruses are spherical or pleomorphic, enveloped viruses, 80 to 120 nm in diameter. Long filamentous forms also occur. The envelope, which is derived from host cell membrane lipids, contains glycosylated and non-glycosylated viral proteins. Surface projections of glycoproteins form 'spikes' or peplomers which, in influenza A and B viruses, are of two types: a haemagglutinin (H) responsible for virus attachment and envelope fusion, and a neuraminidase (N) capable of cleaving viral receptors and promoting both entry of virus into cells and release of virions from infected cells.

Influenza viruses haemagglutinate erythrocytes from a wide range of species. Antibodies to the H glycoprotein are responsible for virus neutralization. The nucleocapsid has a helical symmetry. The genome, which is composed of six to eight segments, consists of linear, negative-sense, single-stranded RNA. Replication occurs in cell nuclei with release of virions by budding from plasma membranes. Virions are labile in the environment and are sensitive to heat, lipid solvents, detergents, irradiation and oxidizing agents.

The family contains four genera, namely *Influenzavirus A*, *Influenzavirus B*, *Influenzavirus C* and *Thogotovirus*. Influenza B and C viruses are pathogens of humans; Thogoto virus and Dhori virus are tick-borne arboviruses isolated from camels, cattle and humans in parts of Africa, Europe and Asia. Influenza A virus, the most important member of the family, is a significant pathogen of animals and humans.

Isolates of influenza A virus are grouped into subtypes on the basis of their H and N antigens. Currently, 15 H antigens and nine N antigens are recognized. New subtypes of influenza A virus emerge periodically. Two mechanisms, point mutation and genetic reassortment, are responsible for the emergence of new strains and new subtypes respectively. Point mutations give rise to antigenic drift, in which variation occurs within a subtype. Genetic reassortment, a more complex process in which the genome segments of two or more related viruses infecting the same cell are exchanged, results in the development of new subtypes (antigenic shift). To assess the risk posed by the emergence of new variant viruses, a precise classification of isolates has been adopted by the World Health Organization. This system is based on the influenza virus type, host, geographical origin, strain number, year of isolation and subtype. An example of this classification system, influenza virus A/equine/Prague/1/56 (H7N7), indicates that this virus was isolated from a horse in Prague during 1956. Antigenic subtypes of influenza A virus which cause disease in humans and animals are presented in Table 56.1.

Clinical infections

Influenza A viruses cause significant infections in humans, pigs, horses and birds. Antibodies to influenza A virus have been detected in cattle in association with respiratory disease, but their significance is unclear. Aquatic birds, particularly ducks which are reservoirs of influenza A virus, provide a genetic pool for the generation of the new subtypes capable of infecting mammals. Migratory waterfowl disseminate the virus across international borders. Although isolates of influenza A virus are usually species specific, there are well documented instances of transfer between species. The viruses replicate in the intestinal tract of birds and transmission is by the faecal-oral route. Human influenza pandemics have been attributed to the combined effects of poor hygiene and the close association of concentrated human populations with domestic fowl and pigs. The frequency of genetic reassortment in these animal populations can lead to the emergence of virulent influenza virus subtypes which are capable of infecting humans, thereby initiating pandemics. Avian influenza viruses usually replicate poorly in humans. However, both human and avian influenza subtypes replicate in pigs, in which species genetic reassortment readily occurs. Because the genome of influenza A virus is segmented, mixed infections frequently give rise to genetic reassortment

Table 56.1 Antigenic subtypes of influenza A virus isolated from humans and animals.

Hosts	Antigenic subtypes	Comments
Humans	H2N8 (1890)[a] H3N8 (1900) H1N1 (1918) H2N2 (1957) H3N2 (1967)	Subtypes which have been found in pigs such as H1N1 have been implicated in human pandemics
Birds	Many antigenic subtypes represented by different combinations of haemagglutinin (H) and neuraminidase (N) peplomers have been recognized	Disease is usually associated with subtypes expressing H5 or H7. Wild birds, especially migrating ducks, act as carriers
Pigs	Predominantly H1N1 and H3N2	Severity of disease is determined by the antigenic subtype
Horses	Usually H7N7 or H3N8	Subtypes associated with disease, which are widely distributed geographically, are absent from Australia, New Zealand and Iceland

a year of recognition

with the emergence of new subtypes. Such novel subtypes are often implicated in major pandemics which occur at about 20 year intervals. As there is limited immunity to the new subtypes in the human population, spread from country to country tends to occur rapidly.

Subtypes of influenza A virus, which are well established as pathogens in animal populations, have also been implicated in crossing species barriers without genetic reassortment. A H1N1 avian subtype appeared in pigs in Europe in 1979. In 1997, following a large epidemic of avian influenza in chickens, a H5N1 subtype was isolated from a fatal case in a young child in Hong Kong. This subtype had not previously been described outside of avian species. Human health fears prompted the destruction of 1.2 million birds in Hong Kong. Fortunately human-to-human transmission did not occur to any significant extent, although other human cases did occur as a result of contact with infected poultry.

Avian influenza (Fowl plague)

Many combinations of H and N antigens in influenza A virus are represented in isolates from avian species, particularly waterfowl. Influenza A virus subtypes are distributed worldwide and are frequently recovered from clinically normal birds. Infection is maintained in wild bird populations. Migrating waterfowl are considered to be responsible for spreading the virus to domestic birds. Outbreaks of severe clinical disease, usually caused by subtypes expressing H5 and H7 determinants, occur periodically in chickens and turkeys. In these species, acute infection is often referred to as fowl plague or highly pathogenic avian influenza and is categorised as a List A disease by the OIE.

Spread of influenza virus in tissues is dependent on the type of proteases present in a given tissue and the structure of the viral haemagglutinin molecule. The production of infectious virions requires cleavage of viral haemagglutinin. In the majority of influenza A virus subtypes, haemagglutinin cleavage takes place in the epithelial cells of the respiratory and digestive tracts. Because of the amino acid composition at their cleavage sites, haemagglutinins of virulent subtypes are susceptible to cleavage in many tissues, facilitating the development of generalized infection. Highly virulent subtypes cause explosive outbreaks of disease with high mortality. Clinical signs are more apparent in birds which survive for a few days. Respiratory distress, diarrhoea, oedema in the cranial region, cyanosis, sinusitis and lacrimation are features of the clinical presentation.

The severe form of the disease may be difficult to distinguish from velogenic viscerotropic Newcastle disease or from fowl cholera. Mild forms of the disease resemble other respiratory conditions in birds. Laboratory confirmation, which involves virus isolation and chacterization, is essential. Definitive subtyping is carried out in reference laboratories using monospecific antisera prepared against the 15 haemagglutinins and nine neuraminidase determinants. Numerous low virulence isolates expressing H5 and H7 determinants have been recorded. To assess pathogenicity, ten chickens should be inoculated intravenously at four to eight weeks of age. Isolates which cause more than 75% mortality within eight days are considered highly pathogenic.

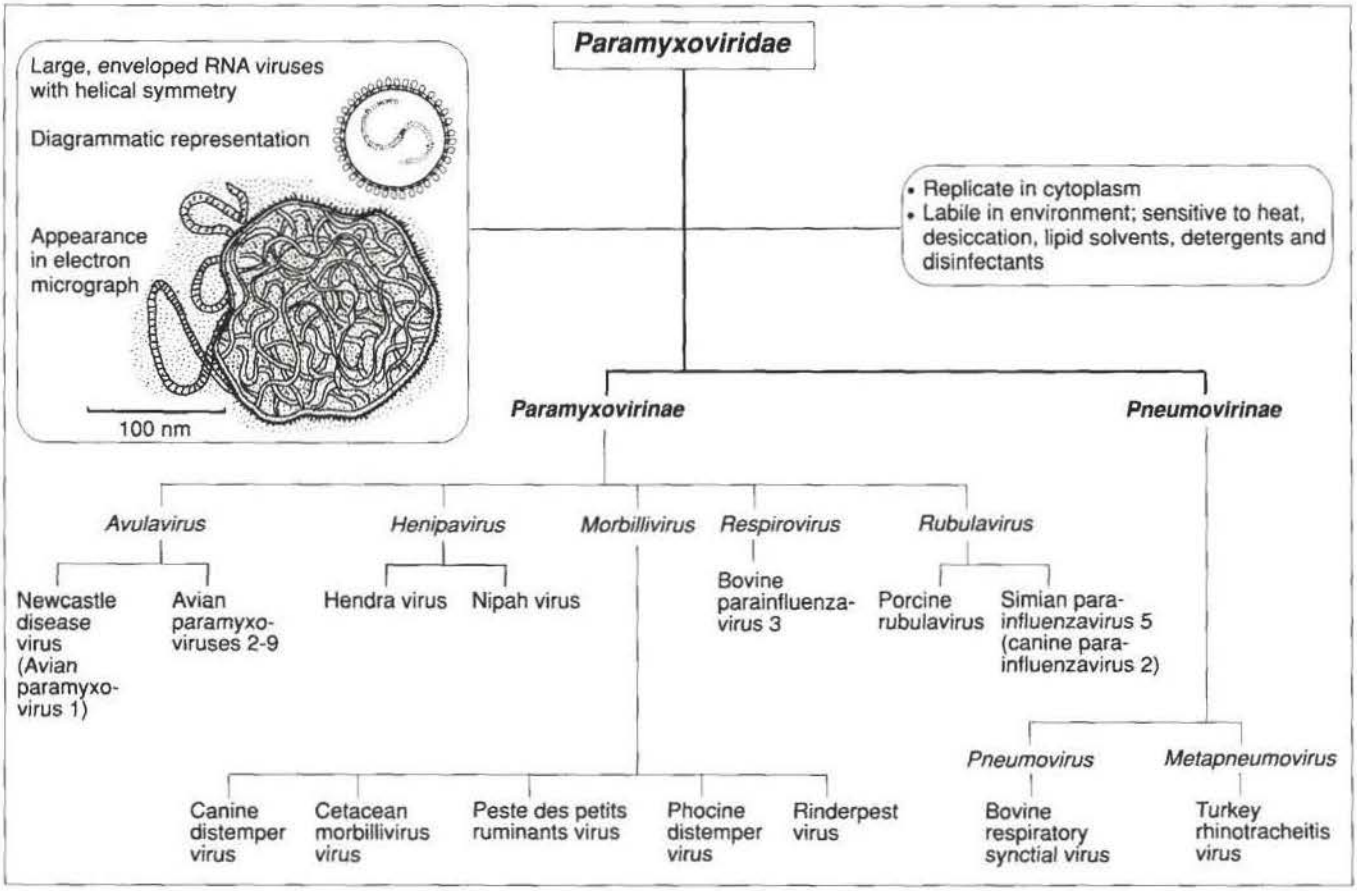
Outbreaks of avian influenza in domestic species are notifiable to national regulatory authorities. In countries free of the disease, outbreaks are controlled by slaughter of affected flocks, imposition of movement restrictions and implementation of rigorous disinfection procedures. Imported birds are quarantined. In high risk areas along the migration routes of waterfowl, poultry should be housed in bird-proof buildings.

Vaccination is usually prohibited in those countries implementing a slaughter policy because of international trade restrictions and possible difficulties in establishing freedom from infection. Some countries accept the presence of mildly pathogenic subtypes because of the expense of implementing control measures. In such countries, inactivated oil emulsion vaccines are available commercially and are used to protect against subtypes of low virulence. Due to the risk of reversion to virulence, live vaccines against influenza A virus are not used.

Equine influenza

Equine influenza is an economically important respiratory disease of horses. Outbreaks of disease are associated with the assembly of horses at shows, sales, racing or training. Affected animals develop a high temperature with nasal discharge and a dry cough. A number of inactivated vaccines are commercially available, but as immunity is short-lived, regular booster injections are required. Vaccinated horses exhibit milder clinical signs than unvaccinated animals.

57 *Paramyxoviridae*



Paramyxoviruses and orthomyxoviruses were formerly grouped together as the 'myxoviruses' (Greek *myxa*, mucus), a name which describes their affinity for mucous membranes. Paramyxoviruses are pleomorphic, 150 nm or more in diameter and enveloped. They contain a single molecule of negative-sense, single-stranded RNA. Two types of glycoprotein 'spikes' or peplomers are present in the envelope; an attachment protein and a fusion protein (F). The attachment protein may either be a haemagglutinin-neuraminidase protein (HN) or a protein without neuraminidase activity (G). The attachment proteins allow the virus to bind to cell surface receptors and the fusion protein causes the virus envelope to fuse with the host cell membrane. Both types of peplomers can induce production of virus neutralizing antibodies. Paramyxoviruses may exhibit haemagglutinating, haemolytic and neuraminidase activities. The nucleocapsid, which has helical symmetry, is 13 to 18 nm in diameter and has a characteristic herring-bone appearance. Replication occurs in the cell cytoplasm. Virions are released by budding from the plasma membrane at sites containing virus envelope proteins. The labile virions are sensitive to heat, desiccation, lipid solvents, non-ionic detergents and disinfectants.

Recently the classification of the *Paramyxoviridae* has been changed to include three new genera, *Metapneumovirus*, *Henipavirus* and *Avulavirus*, and renaming of the genus *Paramyxovirus* as *Respirovirus*. Although paramyxoviruses are genetically stable and do not exhibit recombination, some antigenic variation may occur through mutation.

Clinical infections

Paramyxoviruses, which have a narrow host range, infect mainly mammals and birds (Table 57.1). Following transmission through close contact or by aerosols, replication occurs primarily in the respiratory tract. Disease outbreaks of viral infections in marine mammals has led to the recognition of several new morbilliviruses including phocine distemper virus, dolphin distemper virus and porpoise distemper virus. Hendra virus was isolated during an outbreak of severe respiratory disease in Australia during 1994. Two humans in contact with infected horses were also affected. Fourteen horses and their trainer died. A related virus, Nipah virus, was isolated in Malaysia during 1999 following outbreaks of disease in pigs and humans working in affected pig units. The disease, which caused a febrile encephalitis, resulted in more than 100 human deaths.

Table 57.1 Paramyxoviruses of veterinary importance.

Genus	Virus	Comments
Morbillivirus	Rinderpest virus	Causes highly contagious disease in domestic and wild ruminants, characterized by high morbidity and high mortality
	Peste des petits ruminants virus	Causes severe disease, resembling rinderpest, in small ruminants, particularly sheep and goats, with high morbidity and high mortality rates
	Canine distemper virus	Causes acute disease in dogs and wild carnivores, characterized by multisystemic involvement and variable mortality
Avulavirus	Newcastle disease virus (Avian paramyxovirus 1)	Causes Newcastle disease in domestic and wild birds. Isolates vary in virulence: velogenic, mesogenic and lentogenic strains are recognized. Generalized infection characterized by respiratory, intestinal and nervous signs
Rubulavirus	Porcine rubulavirus	Causes blue eye disease; described only in Mexico
	Canine parainfluenza virus 2	Causes inapparent or mild respiratory disease in dogs; sometimes associated with kennel cough; related to or possibly a subtype of simian virus 5 (SV5)
Respirovirus	Bovine parainfluenza virus 3	Causes subclinical or mild respiratory disease in cattle and sheep. Sometimes associated with shipping fever in cattle. Predisposes to secondary bacterial infection, particularly with *Mannheimia haemolytica*
Pneumovirus	Bovine respiratory syncytial virus	Common subclinical infection in adult cattle. Associated with respiratory disease outbreaks of varying severity in young cattle. Sheep and goats are also susceptible
Metapneumovirus	Turkey rhinotracheitis virus	Causes severe upper respiratory tract infection in turkeys, with coryza and swollen sinuses. In chickens, the disease is referred to as 'swollen head syndrome'

Rinderpest

This acute, List A disease, which occurs primarily in ruminants and is also referred to as cattle plague, has been recognized for centuries as a major cause of mortality in cattle and domestic buffalo. Transmission, which occurs through aerosols, usually requires close contact, as the virus is labile and remains viable in the environment for short periods only. As there is no carrier state, maintenance of infection requires continuous transmission to susceptible animals. Epidemics usually occur following movement of susceptible animals into an endemic area or the introduction of infected animals into susceptible populations. Morbidity may reach 90% and mortality can approach 100%. Infected animals develop a fever and become anorexic and depressed. Mucosal erosions in the mouth and nasal passages become evident within five days . Profuse salivation is accompanied by an oculonasal discharge. About three days after the appearance of the mucosal ulcers, fever regresses and a profuse diarrhoea develops. The dark fluid faeces often contain mucus, necrotic debris and blood. The Food and Agricultural Organization aims to achieve worldwide eradication of rinderpest by 2010. Control of animal movement is the single most important measure in preventing disease transmission.

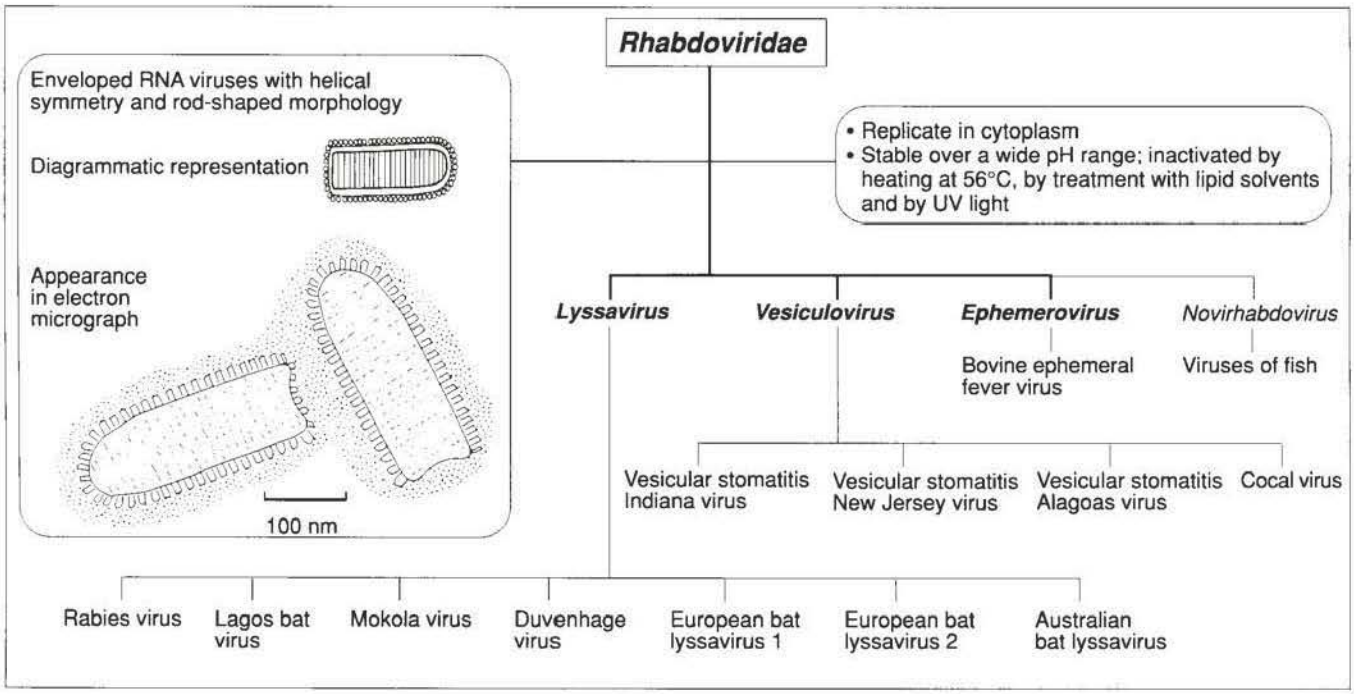
Canine distemper

This highly contagious disease of dogs and other carnivores has a worldwide distribution. Canine distemper virus (CDV), a pantropic morbillivirus, produces a generalized infection involving many organ systems. The virus is relatively labile, requiring transmission by direct contact or by aerosols. Infection spreads rapidly among young dogs, usually between three and six months of age, when maternally-derived immunity declines. The severity and duration of illness are variable and are influenced by the virulence of the infecting virus, the age and immune status of the infected animal and the rapidity of its immune response to infection. Acute disease, which may last for a few weeks, is followed either by recovery and life-long immunity or by the development of neurological signs and, eventually, death. Modified live vaccines, which are available commercially, provide adequate protection when administered to pups after maternally-derived antibody has declined to low levels, usually after 12 weeks of age.

Newcastle disease

This disease occurs in poultry worldwide. A wide range of avian species including chickens, turkeys, pigeons, pheasants, ducks and geese are susceptible. Infection with Newcastle disease virus (NDV) is probably endemic in wild birds, especially waterfowl. Strains of NDV differ in their virulence and isolates are categorized into four pathotypes on the basis of virulence and tissue tropism in poultry. Virus is shed in all excretions and secretions. Transmission usually occurs by aerosols or by ingestion of contaminated feed or water. Respiratory, gastrointestinal and nervous signs occur in chickens. The mortality rate in fully-susceptible flocks may be close to 100%. A combination of vaccination and slaughter is frequently employed to control disease outbreaks.

58 *Rhabdoviridae*



Members of the family *Rhabdoviridae* (Greek *rhabdos*, rod) have characteristic rod shapes. Rhabdoviruses possess a linear, non-segmented RNA genome of negative polarity encased in a ribonucleoprotein complex. It is a large family, containing viruses of vertebrates, invertebrates and plants. Rhabdoviruses of vertebrates are bullet- or cone-shaped, while those infecting plants are generally bacilliform. The family *Rhabdoviridae* comprises six genera. *Vesiculovirus*, *Lyssavirus* and *Ephemerovirus* genera contain viruses which infect vertebrates. Replication occurs in the cytoplasm (with the exception of nucleorhabdoviruses). Newly synthesized nucleocapsids acquire envelopes from the plasma membrane as virions bud from the cell. Virions (100 to 430 nm x 45 to 100 nm) are stable in the pH range of 5 to 10. They are rapidly inactivated by heating at 56°C, by treatment with lipid solvents and by exposure to UV light.

Clinical infections

Rhabdoviruses of veterinary importance are presented in Tables 58.1 and 58.2. They can be transmitted by bites of mammals, arthropod vectors or direct contact. Infection may also be acquired through environmental contamination. The best known and most important member of the *Rhabdoviridae* is rabies virus, a *Lyssavirus* (Greek *lyssa*, rage or fury). A number of distinct *Lyssavirus* genotypes produce clinical signs indistinguishable from rabies. More than 25 viruses isolated from animals have been classified in the genus *Vesiculovirus*. The most important vesiculoviruses which infect domestic animals are the vesicular stomatitis Indiana virus and the vesicular stomatitis New Jersey virus (Table 58.2). Bovine ephemeral fever virus, of significance in some countries, is the type species of the genus *Ephemerovirus*.

Rabies

This viral infection, which affects the central nervous system of most mammals including man, is invariably fatal. However, mammalian species vary widely in their susceptibility. Most clinical cases are due to infection with rabies virus (genotype 1). A number of other neurotropic lyssaviruses, closely related to the rabies virus, produce clinical signs indistinguishable from rabies (Table 58.1). Classical rabies caused by genotype 1 lyssavirus is endemic on continental land masses, with the exception of Australia and Antarctica.

Several species-adapted strains of rabies virus have been described. Strains affecting a particular species are transmitted more readily to members of that species than to other animal species. In a given geographical region, rabies is usually maintained and transmitted by particular mammalian reservoir hosts. Two epidemiologically important infectious cycles are recognised, urban rabies in dogs and sylvatic rabies in wildlife. More than 95% of human cases are the result of bites from rabid dogs. Racoons, skunks, foxes and bats are important reservoirs of rabies virus in North America. In continental Europe, the principal reservoir is the red fox. The vampire bat is an important reservoir of the virus in Central and South America and in the Caribbean islands. Although virus may be transmitted through scratching and licking, transmission usually occurs through bites. Infected animals may excrete virus in their saliva for

Table 58.1 Lyssaviruses which cause rabies and rabies-like diseases.

Virus	Genotype	Serotype	Geographical distribution	Comments
Rabies virus	1	1	Apart from Australia and Antarctica, rabies virus (genotype 1) occurs on all continents. Many island countries are free of the disease	Causes fatal encephalitis in many mammalian species. Transmitted by wildlife species, including foxes, racoons and bats; domestic carnivores also involved in transmission. Rabies is a major zoonotic disease
Lagos bat virus	2	2	Africa	Isolated initially from fruit bats; also isolated from domestic animals with encephalitis
Mokola virus	3	3	Africa	Isolated initially from shrews; also isolated from domestic animals. Human infection reported
Duvenhage virus	4	4	Africa	Originally isolated from a human bitten by an insectivorous bat; additional cases reported in humans. Not reported in domestic animals
European bat lyssavirus 1	5	–	Europe	Identified with increasing frequency in insectivorous bats. Human infection reported
European bat lyssavirus 2	6	–	Europe	Isolated initially from a human with symptoms of rabies; present in insectivorous bats. Additional human cases reported; not reported in domestic animals
Australian bat lyssavirus	7	–	Australia	Identified in fruit bats and in insectivorous bats; human infection reported

Table 58.2 Viruses of veterinary significance in the genera *Vesiculovirus* and *Ephemerovirus*.

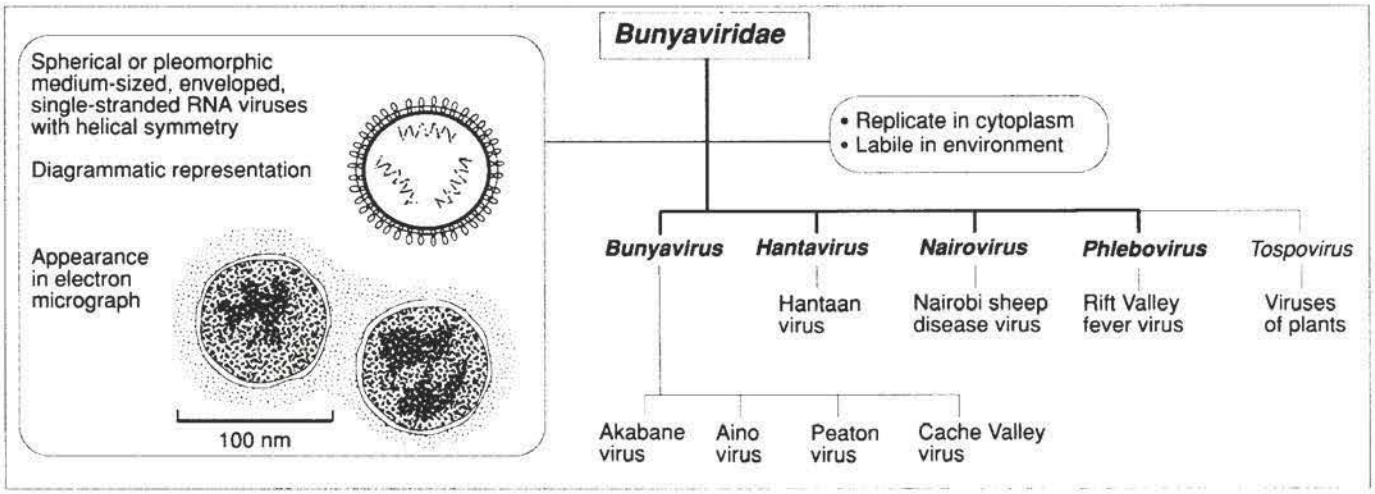
Genus / Virus	Hosts	Comments
Vesiculovirus		
Vesicular stomatitis Indiana virus	Cattle, horses, pigs, humans	Causes febrile disease with vesicular lesions; resembles foot-and-mouth disease clinically. Occurs in North and South America
Vesicular stomatitis New Jersey virus	Cattle, horses, pigs, humans	Causes febrile disease with vesicular lesions; infection more severe than that caused by the Indiana virus. Occurs in North and South America
Vesicular stomatitis Alagoas virus (Brazil virus)	Horses, mules, cattle, humans	Originally isolated from mules in Brazil
Cocal virus (Argentina virus)	Horses	Isolated initially from mites in Trinidad; occurs in South America
Ephemerovirus		
Bovine ephemeral fever virus	Cattle	Causes febrile illness of short duration; occurs in Africa, Asia and Australia

some time before the onset of clinical signs.

The incubation period, which is highly variable and can be as long as six months, is influenced by various factors including host species, virus strain, the amount of inoculum and the site of introduction of the virus. The clinical course in domestic carnivores, which usually lasts for days or for a few weeks, may encompass prodromal, furious (excitative) and dumb (paralytic) phases. Antemortem diagnostic tests for rabies are not generally used. The brains of animals which develop clinical signs should be examined for the presence of virus using the direct fluorescent antibody test. Other methods include demonstration of intracytoplasmic inclusions (Negri bodies) histologically, virus isolation or the reverse transcriptase polymerase chain reaction. Rapid laboratory confirmation is essential for the implementation of appropriate treatment of human patients.

Most countries which are free of rabies rely on rigorous quarantine measures to prevent the introduction of disease. In countries where rabies is endemic, control methods are aimed mainly at reservoir species. Urban rabies can be effectively controlled by vaccination and restriction of movement of dogs and cats and by the elimination of stray animals. Control of sylvatic rabies requires special measures. Vaccination of red foxes with live oral vaccines delivered in baits has eliminated sylvatic rabies from several regions of western Europe. Although attenuated virus vaccines were used initially, there was uncertainty about their ultimate safety. A vaccinia-rabies virus glycoprotein (VRG) vaccine was developed and has proved effective for vaccinating foxes, coyotes and racoons.

59 *Bunyaviridae* and *Birnaviridae*



Bunyaviridae

The family *Bunyaviridae* contains more than 300 viruses. The name of this family is derived from Bunyamwera, the place in Uganda where the type species Bunyamwera virus was first isolated. Virions (80 to 120 nm in diameter) are spherical and enveloped. Glycoprotein peplomers project from the surface of the envelope which encloses three circular, helical nucleocapsid segments. The genome consists of three single-stranded RNA segments designated small (S), medium (M) and large (L). Genetic reassortment occurs between closely-related viruses. The genera in the family are *Bunyavirus*, *Phlebovirus*, *Nairovirus*, *Hantavirus* and *Tospovirus*. Based on antigenic relatedness, viruses within each genus are placed in serogroups. Viruses in the genera *Bunyavirus*, *Phlebovirus*, *Nairovirus* and *Hantavirus* infect vertebrates; those in the genus *Tospovirus* infect plants. Replication takes place in the cytoplasm of host cells. In the final stages of assembly, virions acquire envelopes by budding into the Golgi network. They are then transported through the cytoplasm in secretory vesicles and released by exocytosis at the cell surface. The viruses are sensitive to heat, acid pH levels, lipid solvents, detergents and disinfectants.

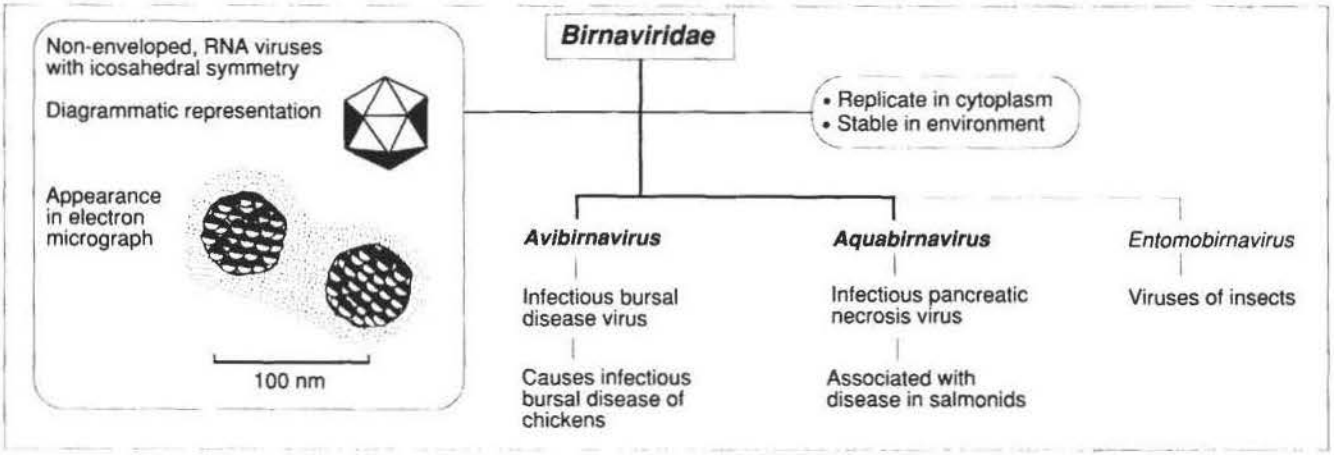
Clinical infections

With the exception of viruses in the genus *Hantavirus*, bunyaviruses are arthropod-borne. These arboviruses are maintained in nature in complex life cycles involving replication in both arthropod vectors and vertebrate hosts. Infection of mammalian cells often results in cytolysis, while infection of invertebrate cells is non-cytolytic and persistent. Mosquitoes are the most important vectors. Ticks, sandflies and midges may act as vectors for some bunyaviruses. Arthropod vectors acquire virus from vertebrate hosts during viraemic periods. Each bunyavirus species replicates in a limited number of vertebrate and invertebrate hosts.

Table 59.1 Bunyaviruses of veterinary importance.

Genus	Virus	Hosts	Comments
Phlebovirus	Rift Valley fever virus	Sheep, cattle, goats	Causes high mortality rates in neonatal animals and abortion in pregnant animals. Endemic in southern and eastern Africa, transmitted by mosquitoes. Important zoonotic disease
Nairovirus	Nairobi sheep disease virus	Sheep, goats	Causes severe, often fatal disease in susceptible animals. Present in central and eastern Africa. Transmitted by ticks
Bunyavirus	Akabane virus, Aino virus, Peaton virus	Cattle, sheep	Viruses belonging to the Simbu serogroup, transmitted by mosquitoes and midges. Widely distributed geographically in tropical and subtropical regions of the Old World. Associated with congenital defects and abortion
	Cache Valley virus	Sheep	Belongs to the Bunyamwera serogroup; transmitted by mosquitoes. Occasionally associated with congenital defects in sheep flocks in North America

Hantaviruses, which are primarily human pathogens, are maintained in nature by persistent infections in rodents which shed virus in urine, faeces and saliva. Transmission between rodent hosts can occur by aerosols and biting. Individual hantaviruses are associated with particular rodent species. Many bunyaviruses infect humans and frequently cause serious diseases, including California encephalitis, haemorrhagic fever with renal syndrome, hantavirus pulmonary syndrome and Crimean-Congo haemorrhagic fever. Such human infections are generally considered to be incidental and do not usually result in disease transmission. Three important ruminant diseases, Rift Valley fever, Nairobi sheep disease and Akabane disease are caused by bunyaviruses (Table 59.1).



Birnaviridae

Birnaviruses are so named because their genomes contain two segments of linear, double-stranded RNA. The icosahedral virions are about 60 nm in diameter. Replication occurs in the cytoplasm of host cells and involves a virion-associated RNA-dependent RNA polymerase. The family *Birnaviridae* contains three genera which infect chickens, fish and insects. Virions are stable over a wide pH range and at a temperature of 60°C for one hour.

Clinical infections

Two economically important diseases associated with birnaviruses are infectious bursal disease of chickens and infectious pancreatic necrosis of salmonids. These diseases occur worldwide and cause considerable losses in poultry units and in farmed salmon.

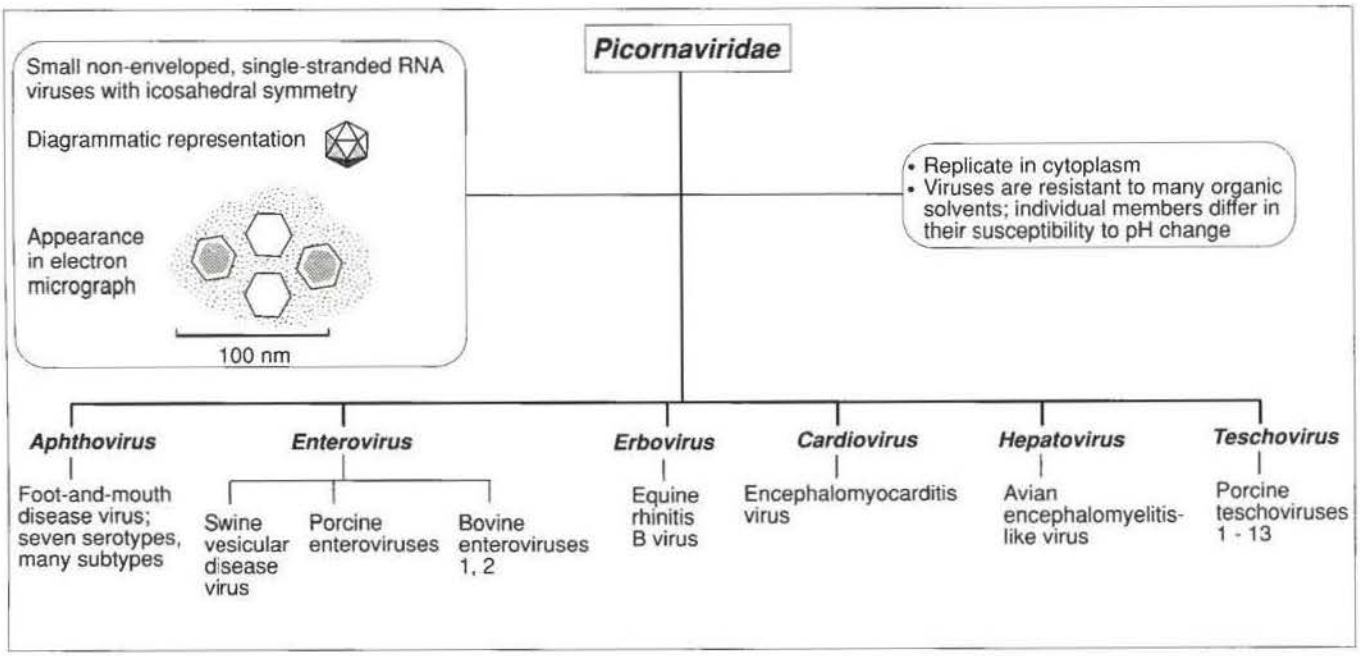
Infectious bursal disease

This condition is a highly contagious disease of young chickens which is caused by infectious bursal disease virus (IBDV). The causal agent was first isolated in Gumboro, Delaware and the disease was originally known as Gumboro disease. Infection, which is usually acquired by the oral route, occurs when maternally-derived antibody levels are waning at two to three weeks of age. Virus is shed in the faeces for up to two weeks after infection and can remain infectious in the environment of a poultry house for several months.

The main target cells are B lymphocytes and their precursors in the bursa. The severity of clinical signs is influenced by the virulence of the virus, the age of chicks at the time of infection, the breed of the chicks and the level of maternally-derived antibody. Chicks develop an acute form of the disease between three and six weeks of age following a short incubation period. Affected birds are depressed and inappetent and show evidence of diarrhoea and vent pecking. Morbidity ranges from 10% to 100% with a mortality rate up to 20% or, occasionally, higher. Many outbreaks are mild, detectable only by impaired weight gains. Although infections before three weeks of age are usually subclinical, severe depression of the humoral antibody response may result. Suboptimal growth, predisposition to secondary infections and poor response to vaccination may be encountered.

Viral antigen can be detected in the bursa using immunofluorescence, ELISA or gel diffusion tests. Depopulation, thorough cleaning and effective disinfection programmes are required following an outbreak of disease in a unit. Most commercial units rely on vaccination for control.

60 *Picornaviridae*



Picornaviruses (Spanish *pico*, very small), which are icosahedral and non-enveloped, contain a molecule of single-stranded RNA. Virions are 30 nm in diameter. The capsid is composed of 60 identical subunits, each containing four major proteins VP1, VP2, VP3 and VP4. The VP4 protein is located on the inner surface of the capsid. Viral replication occurs in the cytoplasm in membrane-associated complexes and infection is usually cytolytic. The family comprises six genera: *Enterovirus*, *Rhinovirus*, *Cardiovirus*, *Aphthovirus*, *Hepatovirus* and *Parechovirus*. In addition three new genera: *Erbovirus*, *Kobuvirus* and *Teschovirus*, have recently been established. Several enteroviruses of pigs and poultry have been reassigned. Porcine enteroviruses 1-7 and 11-13, which are associated with nervous disease and reproductive problems in pigs, are being reassigned to the genus *Teschovirus*. Avian encephalomyelitis-like virus is now considered a member of the genus *Hepatovirus*.

Viruses of veterinary importance in the family *Picornaviridae* are presented in Table 60.1. Picornaviruses are resistant to ether, chloroform and non-ionic detergents. Individual genera differ in their thermal lability and pH stability. Apthoviruses are unstable at pH values below 6.5 and rhinoviruses are unstable below pH 5.0. Viruses in the other genera are stable at acid pH values. Some viruses in the genera *Hepatovirus* and *Parechovirus*, including hepatitis A virus, are important human pathogens. Poliomyelitis virus, which causes serious neurological disease in humans, is an enterovirus.

Clinical infections

With the exception of foot-and-mouth disease virus and encephalomyocarditis virus, picornaviruses typically infect a single, or a limited number of, host species. Transmission usually occurs by the faecal-oral route but may also occur by fomites or by aerosols. Some picornaviruses, notably foot-and-mouth disease virus and swine vesicular disease virus, can produce persistent infections. Antigenic variation, which may contribute to the development of persistent infection, has been attributed to a number of molecular mechanisms, including genetic recombination. Mixed infections with different serotypes of foot-and-mouth disease virus are known to occur in individual animals, particularly in African Cape buffaloes. Although infections with enteroviruses are common in many vertebrate species, significant disease occurs only in pigs, poultry and humans. Rhinoviruses, which are associated with the common cold in humans, are considered to be minor pathogens in cattle.

Foot-and-mouth disease

This highly contagious disease of even-toed ungulates is characterized by fever and the formation of vesicles on epithelial surfaces. Foot-and-mouth disease (FMD) is a List A disease of major importance internationally on account of its rapid spread and the dramatic economic losses which it causes in susceptible animals. Isolates of foot-and-mouth disease virus (FMDV) are grouped in seven serotypes, recognised as separate species,

Table 60.1 Picornaviruses of veterinary importance.

Genus	Virus	Comments
Enterovirus	Swine vesicular disease virus	Produces mild vesicular disease, clinically indistinguishable from foot-and-mouth disease
	Bovine enteroviruses 1 and 2	Isolated from both normal cattle and animals with enteric, respiratory and reproductive disease
Teschovirus	Porcine teschovirus 1	Virulent strains which occur in eastern Europe and the Malagasy Republic cause severe encephalomyelitis (Teschen disease); widely distributed mild strains cause endemic posterior paresis (Talfan disease)
Hepatovirus	Avian encephalomyelitis-like virus	Avian encephalomyelitis is of considerable economic importance in chickens. Horizontal and vertical transmission occurs. Nervous signs seen in birds at 1 to 2 weeks of age. Control is achieved by vaccination of breeding flocks
Aphthovirus	Foot-and-mouth-disease virus	Seven serotypes are recognised: A, O, C, Asia 1, SAT 1, SAT 2, SAT 3. Economically important, highly contagious vesicular disease of even-toed ungulates
Cardiovirus	Encephalomyocarditis virus	Wide host range; rodents are considered to be the natural hosts. Infection in pigs is often subclinical but sporadic deaths and minor outbreaks may occur

with differing geographical distributions. Infection with one serotype does not confer immunity against the other serotypes. A large number of subtypes is recognized within each serotype.

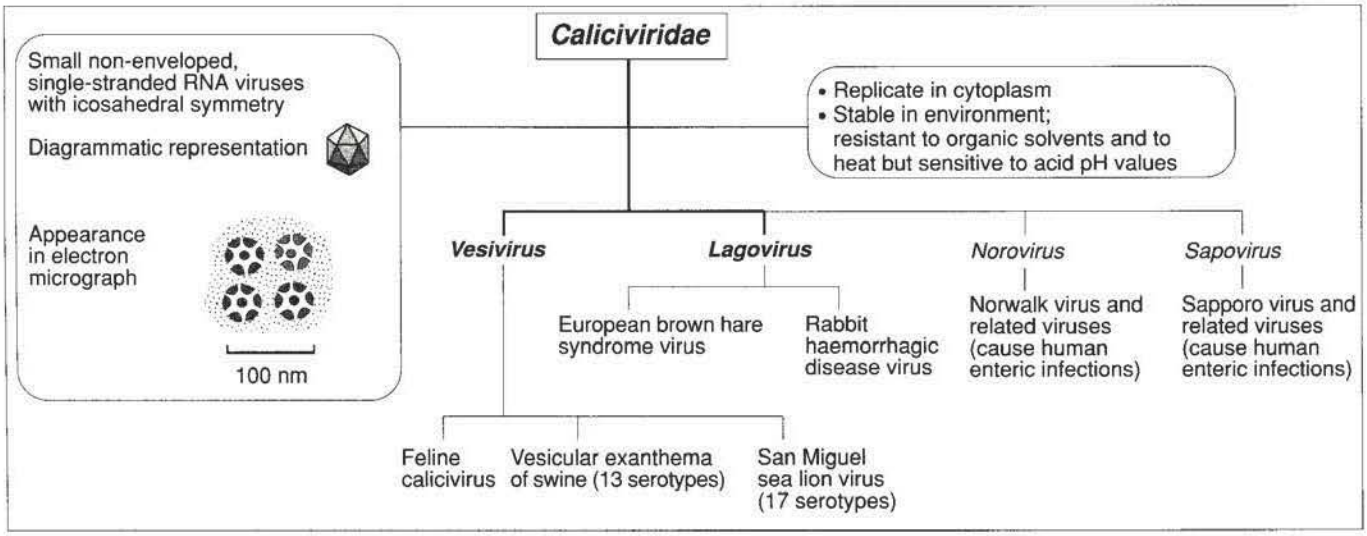
Cattle, sheep, goats, pigs and domesticated buffaloes are susceptible to FMD. Several wildlife species including African buffaloes, elephants, hedgehogs, deer and antelopes are also susceptible. Large numbers of virus particles are shed in the secretions and excretions of infected animals. Transmission can occur by direct contact, by aerosols, by mechanical carriage by humans or vehicles, on fomites and through animal products. Infected groups of animals, particularly pigs, shed large quantities of virus in aerosols. Under favourable conditions of low temperature, high humidity and moderate winds, virus in aerosols may spread up to 10 km over land. Turbulence is generally less marked over water than over land. In 1981, virus was carried a distance of more than 200 km from France to the south coast of England. Foot-and-mouth virus can persist in the pharyngeal region of carrier animals which have recovered from FMD.

The incubation period ranges from 2 to 14 days, but is generally shorter than a week. Infected cattle develop fever, inappetence and a drop in milk production. Profuse salivation, with characteristic drooling and smacking of lips, accompanies the formation of oral vesicles which rupture, leaving raw, painful ulcers. Ruptured vesicles in the interdigital cleft and on the coronary band lead to lameness. Vesicles may also appear on the skin of the teats and udders of lactating cows. Although the ulcers tend to heal rapidly, there may be secondary bacterial infection which exacerbates and prolongs the inflammatory process. Infected animals lose condition. Mature animals seldom die. Calves may die from acute myocarditis. In pigs, foot lesions are severe and the hooves may slough. Marked lameness is the most prominent sign in this species. The disease in sheep, goats and wild ruminants is generally mild, presenting as fever accompanied by lameness which spreads rapidly through groups of animals.

Foot-and-mouth disease clinically resembles other vesicular diseases of domestic animals, including vesicular stomatitis in cattle and pigs, swine vesicular disease and vesicular exanthema in pigs. Consequently, FMD requires laboratory confirmation. Diagnosis is based on the demonstration of FMDV antigen in samples of tissue or in vesicular fluid by ELISA, CFT, RT-PCR or virus isolation. Demonstration of specific antibody by virus neutralization or ELISA can be used to confirm a diagnosis in unvaccinated animals. In endemic areas, interpretation of antibody titres may prove difficult.

In countries which are free from FMD, it is a notifiable disease and affected and in-contact animals are slaughtered. Following an outbreak, movement restrictions are applied and infected premises must be thoroughly cleaned and disinfected. Mild acids, such as citric acid and acetic acid, and alkalis such as sodium carbonate are effective disinfectants. Reserves of inactivated virus are maintained in several countries to provide an adequate supply of vaccine at short notice in the event of a major outbreak of the disease. Although ring vaccination around an affected premises may help to limit the spread of the disease, it may also allow the development of the carrier state in animals subsequently exposed to the virus. In countries where FMD is endemic, efforts are generally directed at protecting high-yielding dairy cattle by a combination of vaccination and control of animal movement. Vaccines for FMD, incorporating adjuvant, are derived from tissue culture-propagated virus which has been chemically inactivated. They are usually multivalent, containing three or more virus strains. Protection against antigenically similar strains of virus is satisfactory and lasts for up to six months.

61 *Caliciviridae*



Caliciviruses (Latin *calix*, cup) have cup-shaped depressions, demonstrable by electron microscopy, on the surface of virions. The virions, 27 to 40 nm in diameter, are icosahedral and non-enveloped. The genome consists of a single molecule of linear, positive-sense, single-stranded RNA. Replication takes place in the cytoplasm of infected cells and virions are released by cell lysis. Many caliciviruses have not yet been cultured. The virions are resistant to ether, chloroform and mild detergents. They are relatively resistant to heat but are sensitive to acid pH values.

Caliciviruses, which are closely related to picornaviruses, were formerly grouped within the *Picornaviridae*. Currently, the family *Caliciviridae* is divided into four genera: *Vesivirus*, *Lagovirus*, *Norovirus* and *Sapovirus*. The members of the latter two genera are human caliciviruses which cause gastroenteritis.

Clinical infections

Caliciviruses have been recovered from many species including humans, cats, pigs, marine mammals, rabbits, hares, cattle, dogs, reptiles, amphibians, shellfish and insects. They are associated with a wide range of conditions including respiratory disease, vesicular lesions, necrotizing hepatitis and gastroenteritis (Table 61.1). Infections with caliciviruses, which are frequently persistent, may be inapparent, mild or acute. Transmission occurs directly or indirectly without vector involvement.

Feline calicivirus infection

Infections caused by feline calicivirus (FCV) account for about 40% of upper respiratory tract inflammatory disease in cats worldwide. All species of *Felidae* are considered to be susceptible but natural disease tends to be confined to domestic cats and to cheetahs in captivity. There is a high degree of antigenic heterogeneity among FCV isolates. Sequence analysis studies have shown that individual isolates of FCV exist as quasi-species which evolve and exhibit antigenic drift. Significant alterations in the antigenic profiles of sequential virus isolates from carrier cats are thought to be influenced by immune selection and may play an important part in viral persistence.

Virus replication occurs primarily in the oropharynx with rapid spread throughout the upper respiratory tract and to the conjunctivae. A transient viraemia occurs. Infections range from subclinical to severe, reflecting differences in strain virulence. Virulent strains of FCV can cause interstitial pneumonia in young kittens. The virus has been recovered from the joints of lame cats.

The incubation period is up to five days. Clinical signs, which are usually confined to the upper respiratory tract and the conjunctivae, are often less severe than those caused by feline herpesvirus 1 infection. Fever, oculonasal discharge and conjunctivitis are accompanied by the development of characteristic vesicles on the tongue and oral mucosa. These vesicles rupture leaving shallow ulcers. Morbidity may be high but mortality is usually low. Stiffness and shifting lameness, which usually resolve within a few days, are sometimes seen during the acute phase of FCV infection or following inoculation with FCV vaccine. An association between infection with FCV and chronic gingivitis and stomatitis, when infection with feline immunodeficiency virus is also present, has been suggested.

Although cats of all ages are susceptible to infection with FCV, acute disease occurs most commonly in kittens as maternally-derived antibody wanes between two and three months of age. Infected cats excrete large amounts of virus in oronasal secretions. Many cats remain persistently infected after recovery from acute infection, following subclinical infection while protected by maternally-derived antibody or by vaccination. Infection is maintained in the cat population by these carriers

Table 61.1 Caliciviruses of veterinary importance.

Virus	Hosts	Comments
Vesicular exanthema of swine virus (13 serotypes)	Pigs	Acute, contagious vesicular disease, clinically similar to foot-and-mouth disease. Occurred in the USA before 1956. May have arisen from feeding sea lion and seal meat contaminated with San Miguel sea lion virus
San Miguel sea lion virus (17 serotypes)	Marine mammals, Opal eye fish	Associated with cutaneous vesicles and premature parturition in pinnipeds; when inoculated into pigs, causes vesicular exanthema
Feline calicivirus	Domestic and wild cats	Important cause of upper respiratory tract infection in cats worldwide
Rabbit haemorrhagic disease virus	European rabbits	Acute fatal disease in European rabbits over two months of age
European brown hare syndrome virus	European brown hare	Related to rabbit haemorrhagic disease virus. Causes hepatic necrosis and widespread haemorrhages with high mortality
Canine calicivirus	Dogs	Occasionally associated with diarrhoea

which shed virus continuously from the oropharynx for months and, occasionally, for years.

As the clinical signs are similar, differentiation from feline herpesvirus 1 infection requires laboratory testing. Feline calicivirus can be isolated in feline cell lines from oropharyngeal swabs or from lung tissue. However, isolation of FCV may not be aetiologically significant because of the large numbers of carrier animals in cat populations. Demonstration of a rising antibody titre in paired serum samples is required for laboratory confirmation.

Vaccination and management practices aimed at reducing exposure to the virus are the main methods of control. Inactivated vaccines for parenteral administration and modified live vaccines for either parenteral or intranasal administration are available. Although vaccination protects effectively against clinical disease, it does not prevent subclinical infection or the development of a carrier state. Vaccines are based on a limited number of FCV isolates which cross-react with a broad spectrum of field isolates. Live vaccines, for administration by injection, may cause clinical signs if given by other routes.

Rabbit haemorrhagic disease

This is a highly contagious, acute and often fatal disease of European rabbits (*Oryctolagus cuniculus*). Rabbit haemorrhagic disease (RHD) was first reported in China in 1984 and has since been encountered in many parts of the world. This virus (RHDV) is considered to be a mutant form of a non-pathogenic virus, termed rabbit calicivirus, which has been endemic in commercial and wild rabbits in Europe for many years. Rabbit haemorrhagic disease virus has been used for biological control of rabbits in Australia and New Zealand.

Virus is shed in all excretions and secretions. Among rabbits in close contact, transmission is mainly by the faecal-oral route. Infection may also occur by inhalation or through the conjunctiva. Mechanical transmission by a variety of insects, including mosquitoes and fleas, has been demonstrated. The virus survives in the environment and indirect transmission through contaminated foodstuffs or fomites may occur.

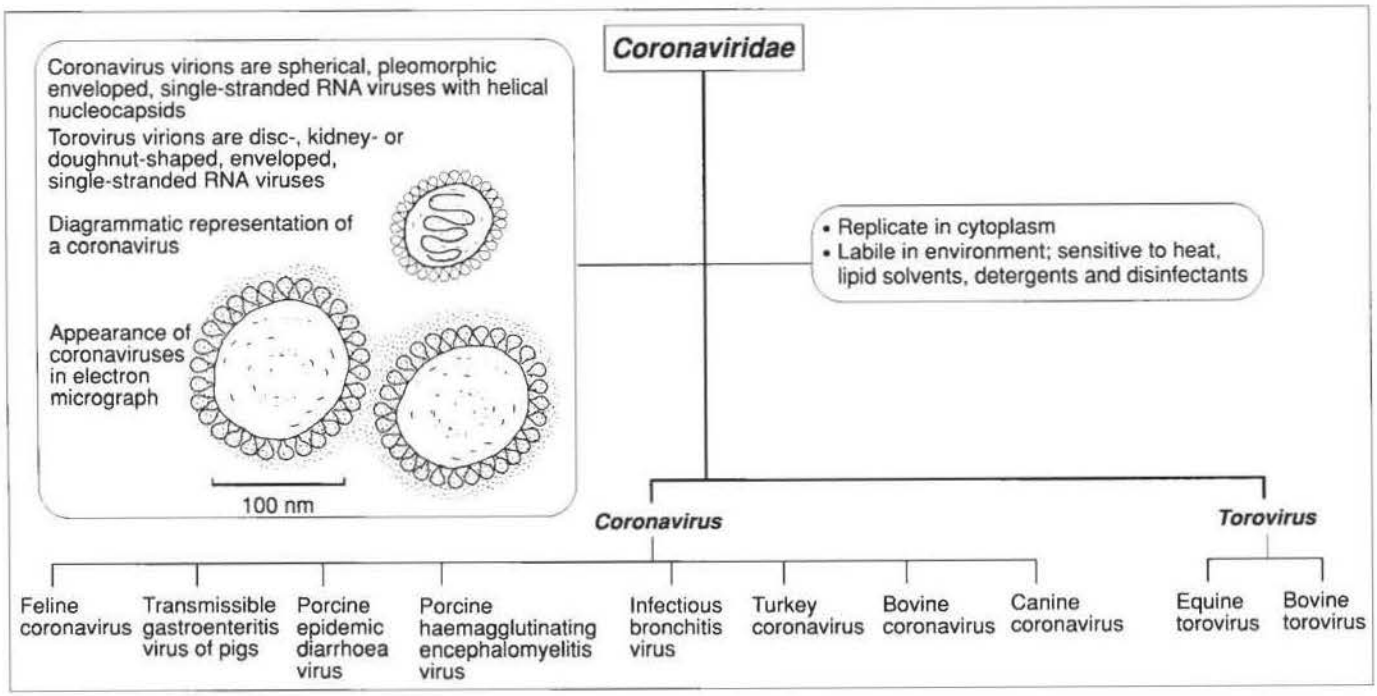
Cells of the mononuclear phagocyte lineage are considered to be the major targets of the virus. Rabbits under two months of age do not develop clinical signs. The reason for this resistance is unclear, but it may have a physiological basis. Severe hepatic necrosis is the most obvious lesion in affected rabbits. In addition, there may be evidence of disseminated intravascular coagulation.

The incubation period is up to three days. The disease is characterized by high morbidity and high mortality. The course is short, with death occurring within 36 hours of the onset of clinical signs. Acutely affected animals are pyrexic and depressed and have an increased respiratory rate. A serosanguineous nasal discharge, haematuria and neurological signs, including convulsions, may be present. Rabbits may be found dead or die in convulsions. A few rabbits may present with milder, subacute signs during the later stages of a major outbreak. Some animals may survive for a few weeks with jaundice, weight loss and lethargy.

High mortality in rabbits along with characteristic gross lesions including necrotic hepatitis and congestion of spleen and lungs are suggestive of RHD. Culture of RHDV has been unsuccessful. High concentrations of virus are present in affected livers. Confirmation is based on detection of virus by electron microscopy or of viral antigen by ELISA, immunofluorescence or haemagglutination using human erythrocytes. Suitable serological tests for the detection of specific antibodies to the virus include haem-agglutination-inhibition and ELISA. Reverse transcriptase PCR has been developed for the detection of RHDV nucleic acid.

In countries where RHD is endemic, control is achieved by vaccination. Inactivated and adjuvanted vaccines prepared from clarified liver suspensions of experimentally-infected rabbits are usually administered at about 10 weeks of age.

62 *Coronaviridae*



Members of the family *Coronaviridae* (Latin *corona*, crown) are large, pleomorphic, enveloped viruses. They contain a single molecule of linear, positive-sense, single-stranded RNA. Club-shaped glycoprotein peplomers projecting from the envelope impart a crown-like appearance to the virus. Each peplomer is composed of a large viral glycoprotein (spike or S protein) which is responsible for attachment to cells. The S protein is the main antigenic component which induces the production of neutralizing antibodies during natural infection. Hypervariable domains in the S protein facilitate the production of virus escape mutants, capable of evading the host immune response. There are two genera in the family, *Coronavirus* and *Torovirus*. Coronaviruses, which are almost spherical with a diameter of 120 to 160 nm, have helical nucleocapsids. Toroviruses, which have a tubular nucleocapsid, may be disc-shaped, kidney-shaped or rod-shaped and are 120 to 140 nm in diameter.

Coronaviruses replicate in the cytoplasm of cells. Newly synthesized virions acquire their envelopes from the membranes of the endoplasmic reticulum and the Golgi complex. Genetic recombination can occur at high frequency between related coronaviruses.

With the exception of infectious bronchitis virus, coronaviruses are usually difficult to grow in cell culture. The virions are sensitive to heat, lipid solvents, formaldehyde, oxidizing agents and non-ionic detergents. The stability of coronaviruses at low pH values is variable.

Clinical infections

Coronaviruses can infect a number of mammalian and avian species and many display tropisms for respiratory and intestinal epithelium. The coronaviruses of veterinary importance and the clinical consequences of infection are indicated in Table 62.1. Feline coronavirus, canine coronavirus and transmissible gastroenteritis virus are closely related antigenically. Transmissible gastroenteritis (TGE) is a highly contagious coronaviral disease of young pigs which occurs worldwide. The virus which causes TGE is stable at low pH values. Coronaviral infections are usually mild or inapparent in mature animals but may be severe in young animals. Coronaviruses are aetiologically important in humans as a cause of the common cold and, more recently, have been implicated in a new respiratory disease termed severe acute respiratory syndrome (SARS).

Although evidence of torovirus infection has been found in pigs, sheep, goats and cats, the clinical significance of these infections is questionable. Two toroviruses have been implicated in enteric diseases of domestic animals, equine torovirus (Berne virus) and bovine torovirus (Breda virus).

Feline infectious peritonitis

Feline infectious peritonitis (FIP), caused by certain strains of feline coronavirus, is a worldwide and invariably fatal, sporadic disease of domestic cats and other *Felidae*. Strains of feline coronavirus vary in pathogenicity. The term feline enteric coronavirus (FECV) has been used to describe strains that cause

Table 62.1 Coronaviruses of veterinary significance.

Virus	Consequences of infection
Feline coronavirus (FCoV)	Replicates in enterocytes; subclinical infection common. May produce mild gastroenteritis in young kittens; also referred to as feline enteric coronavirus (FECV). Feline infectious peritonitis virus (FIPV) is considered to have derived from strains of FCoV which initially replicated in enterocytes and subsequently in macrophages; causes sporadic fatal disease of young cats, often presenting clinically as an effusive peritonitis
Transmissible gastroenteritis virus (TGEV)	Highly contagious infection with vomiting and diarrhoea in piglets; high mortality in newborn piglets. A deletion-mutant of TGEV, porcine respiratory coronavirus, induces partial immunity to TGEV
Porcine epidemic diarrhoea virus	Causes enteric infection similar to that caused by TGEV but with lower neonatal mortality
Porcine haemagglutinating encephalomyelitis virus	Nervous disease or vomiting and emaciation (vomiting and wasting disease) in young pigs. Infection is widespread but clinical disease is uncommon
Infectious bronchitis virus	Acute, highly contagious respiratory infection in young birds; causes a drop in egg production in layers
Turkey coronavirus	Infectious enteritis (bluecomb disease)
Bovine coronavirus	Diarrhoea in calves; associated with winter dysentery in adult cattle
Canine coronavirus	Asymptomatic infection or diarrhoea in dogs

mild or inapparent enteritis, while the term feline infectious peritonitis virus (FIPV) was applied to those strains aetiologically implicated in FIP. It is believed that FIPV occurs as a mutant of the widely distributed FECV, resulting in an alteration in tropism from enteric epithelial cells to macrophages. The term feline coronavirus (FCoV), as currently used, includes all strains irrespective of virulence.

Feline infectious peritonitis occurs sporadically in catteries or multicat households. The incidence is reported to be higher in pedigree cats. Although cats of any age may be affected, those less than one year of age appear to be most susceptible. Infected cats shed virus in faeces and oronasal secretions. Transmission is mainly by ingestion or inhalation. Infection is acquired by young kittens from their mothers or from other adult cats. Infection with FIPV does not always result in clinical disease. Factors which may influence the development of the disease include the age, immune status and genetic characteristics of the host and the emergence of virulent virus strains. In some instances, probably due to mutational changes in the virus, the emergence of a virulent FIPV strain results in systemic invasion with replication in macrophages. In most infected kittens, the development of effective cell-mediated immunity (CMI) restricts viral replication and ultimately eliminates infection. Some individual animals with less effective CMI may shed virus intermittently while remaining clinically normal. When CMI is severely impaired or defective, virus replication continues, leading to B cell activation and the production of non-protective antibodies. The immune complexes, formed from these antibodies and FIPV, activate complement leading to immune-mediated vasculitis.

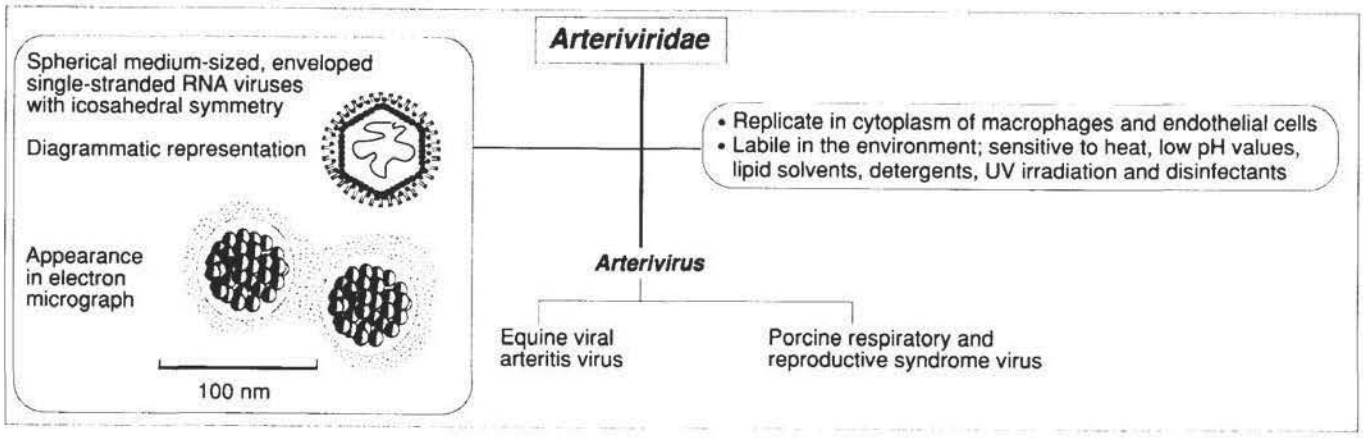
The incubation period ranges from weeks to months. The onset of clinical signs may be either sudden or slow and insidious. Early signs, which are generally non-specific, include anorexia, weight loss, listlessness, dehydration and icterus. Cats with the effusive form of the disease have fibrin-rich exudates in the abdominal or thoracic cavities. If the pleural effusion is marked, dyspnoea develops. The effusive form of the disease usually leads to death within eight weeks. In the non-effusive form of FIP, clinical findings are less characteristic. Signs referable to lesions in organs or tissues in the abdominal cavity are present in about 50% of affected cats. Anterior uveitis, chorioretinitis and neurological signs may be evident in up to 30% of cases.

Currently, histological examination of affected tissues is the only procedure available for the definitive diagnosis of FIP. A serum hyperproteinaemia is frequently present in affected cats due to a hypergammaglobulinaemia. Diagnostic serological tests, including IFA and ELISA do not distinguish between cats infected with FCoV and FIPV. An intranasal vaccine employing a temperature-sensitive mutant strain of FIPV has recently been developed. Although some efficacy and safety test results have been favourable, other studies have failed to demonstrate significant protective immunity.

Infectious bronchitis

Infectious bronchitis, caused by infectious bronchitis virus, is a highly contagious, economically important, worldwide disease of poultry which affects the respiratory, reproductive and renal systems. The chicken is the main host and the most important route of transmission is by aerosols. Spread of infection occurs rapidly among susceptible birds and mortality may reach 100%. The respiratory system is the primary site of viral replication. The disease , which has a short incubation period, is most severe in young birds. In chickens less than three weeks of age, gasping and nasal exudate are prominent clinical signs. In older birds, rales and gasping may be observed. Both live and killed vaccines are available. Live vaccines are usually given in drinking water or by aerosol to chickens up to 14 days of age and again at about four weeks of age.

63 *Arteriviridae* and *Togaviridae*



Arteriviridae

Arteriviruses, formerly classified as members of the family *Togaviridae*, have recently been assigned to the family *Arteriviridae* which contains a single genus, *Arterivirus*. The name of the genus derives from the disease, equine arteritis, which is caused by the type species. Arteriviruses are spherical, 40 to 60 nm in diameter and possess a lipid-containing envelope which has ring-like surface structures. The icosahedral nucleocapsid contains a molecule of linear single-stranded RNA. Replication takes place in the cytoplasm of infected cells. Arteriviruses, which are relatively labile, are sensitive to heat, low pH, lipid solvents, detergent treatment, UV irradiation and many disinfectants.

Clinical infections

Members of the genus are host-specific and antigenically unrelated. Infections have been described in horses, pigs, mice and monkeys. The primary target cells are macrophages. Infection is spread horizontally by aerosol, by biting or by venereal transmission. Infections are frequently persistent.

Equine viral arteritis

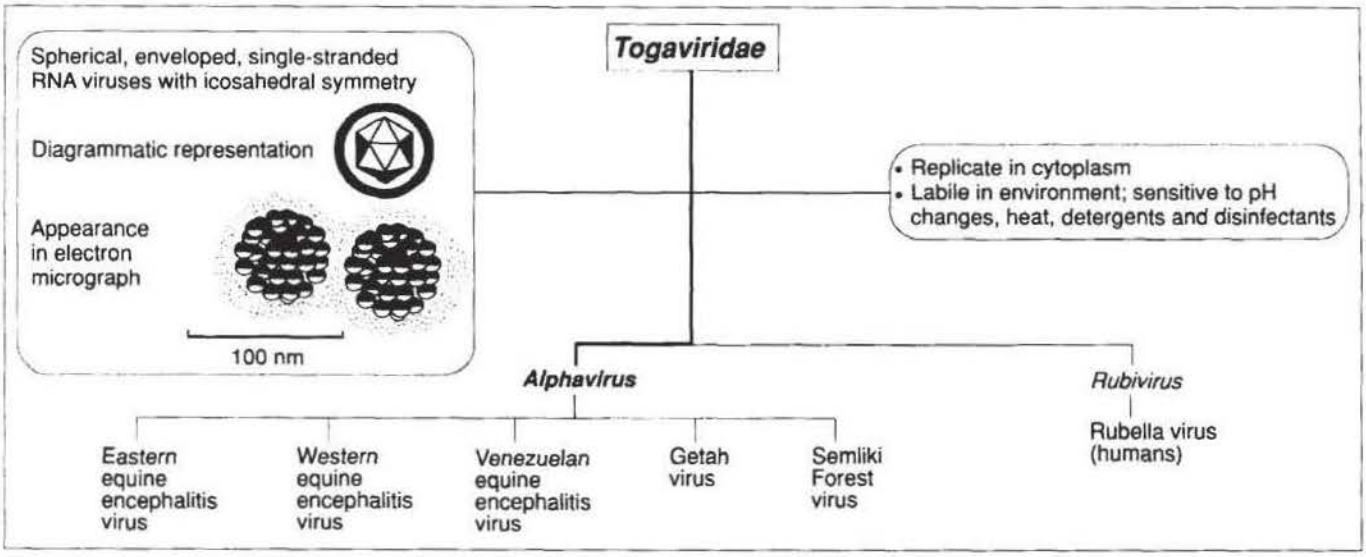
Although infection with equine arteritis virus (EAV) occurs worldwide, outbreaks of clinical disease are comparatively rare. Upper respiratory tract infection, ventral oedema and abortion are prominent clinical features. During the acute phase of infection, virus is spread primarily by aerosols from the respiratory tract. Virus is also shed in faeces, urine and vaginal secretions. Close contact facilitates spread of infection. Virus is usually eliminated from mares and geldings within one to two months but may persist in about 35% of infected stallions. Carrier stallions are asymptomatic and shed virus continuously in semen. Mares infected venereally may spread virus horizontally to in-contact susceptible animals. Abortion or infection of the foal may occur when pregnant mares are infected.

Diagnosis requires laboratory confirmation. Virus isolation should be carried out in appropriate cell lines such as rabbit or equine kidney cells. Viral RNA can be detected in semen and other specimens using the reverse transcriptase polymerase chain reaction. Acute and convalescent blood samples should be submitted for serology. Carrier stallions can be identified by serological testing. Persistently-infected stallions should be identified and their breeding activities confined to seropositive or vaccinated mares. In order to reduce the risk of colt foals becoming carriers, vaccination at six to 12 months of age is recommended.

Porcine respiratory and reproductive syndrome

This economically important condition is characterized by reproductive failure in sows and pneumonia in young pigs. The syndrome was first described in the USA in 1987. Despite attempts at controlling spread, the disease is now endemic in many countries. Natural infection occurs in pigs and wild boars. Virus, which is shed in saliva, urine, semen and faeces, is highly infectious. Nose-to-nose contact is considered to be the most likely route of transmission. Introduction of porcine respiratory and reproductive syndrome virus (PRRSV) to a breeding herd is usually followed by reproductive failure which may take the form of abortions, early farrowing, increased numbers of stillborn and mummified foetuses, weak neonatal pigs and delayed return to service in affected sows. A 'rolling inappetence', progressively affecting animals in an infected herd, has been described. In some cases, cyanosis of the ears and vulva along with erythematous plaques on the skin ('blue-eared disease') have been described. Respiratory distress and increased preweaning mortality are important features of the disease in neonatal pigs. Subclinical infection is common.

Serology is the most widely used diagnostic method. However, these tests do not distinguish carrier from vaccinated animals. The presence of PRRSV may be demonstrated by virus isolation, direct FA staining, *in situ* hybridization or reverse transcriptase polymerase chain reaction. Vaccination and effective hygiene and health management are important for controlling infection.



Togaviridae

Viruses in the family *Togaviridae* (Latin *toga*, cloak) are enveloped RNA viruses, approximately 70 nm in diameter, with icosahedral symmetry. The envelope, which contains glycoprotein spikes, is closely bound to an icosahedral capsid. There are two genera, *Alphavirus* and *Rubivirus*, in the family. The sole member of the genus *Rubivirus* is rubella virus, which causes German measles in children and young adults.

The genus *Alphavirus* includes more than 25 species, a number of which are important animal pathogens. Alphaviruses are divided, on the basis of genomic composition, into a number of groups including Venezuelan equine encephalitis virus (VEEV) complex, eastern equine encephalitis virus (EEEV) complex, Semliki Forest virus complex and western equine encephalitis virus (WEEV) complex. Western equine encephalitis virus has been shown to have arisen by recombination between EEEV and Sindbis-like viruses, probably between 1,300 and 1,900 years ago.

Replication of alphaviruses, which contain positive-sense single-stranded RNA, occurs in the cytoplasm and nucleocapsids are assembled in the cytosol. In vertebrates, alphavirus infection results in cytolysis. The viral envelope is acquired as the nucleocapsid buds into the plasma membrane which contains virus-derived glycoprotein spikes. Viral infection of invertebrate cells is usually non-cytolytic and persistent. In this instance virus assembly occurs in association with intra-cellular membranes rather than through the plasma membrane.

Mature virions of alphaviruses are sensitive to pH changes, heat, detergents and disinfectants, and are not stable in the environment. Alphaviruses, in common with certain members of the *Flaviviridae*, *Reoviridae*, *Rhabdoviridae* and *Bunyaviridae*, are termed arboviruses indicating that they are arthropod-borne. This term, however, has no taxonomic significance.

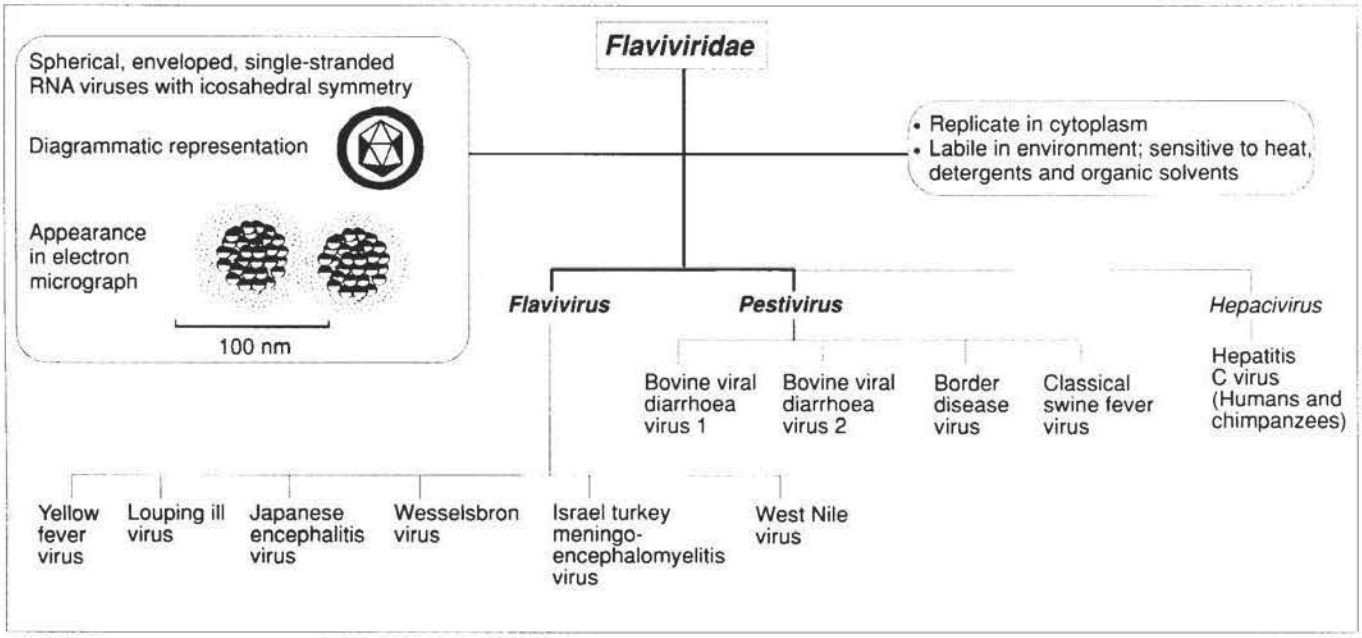
Clinical infections

Domestic animals and humans are usually considered to be 'dead-end' hosts of alphaviruses because they do not develop a sufficiently high titre of circulating virus to act as reservoir hosts. A number of important equine diseases are caused by infection with members of the genus *Alphavirus* (Table 63.1). The three equine encephalitis viruses (Venezuelan, eastern and western), which are confined to the western hemisphere, are transmitted by mosquitoes. Getah virus occurs mainly in south-east Asia and in Australia. A number of outbreaks of disease caused by this virus have been recorded in Japan.

Table 63.1 Alphaviruses of veterinary significance.

Virus	Vector	Comments
Eastern equine encephalitis virus	Mosquito (*Culiseta melanura*, *Aedes* species)	Infection endemic in passerine birds which frequent freshwater swamps of eastern North America, Caribbean islands and parts of South America. Causes disease in horses, humans and pheasants
Venezuelan equine encephalitis virus	Mosquito (*Culex* species)	Infection endemic in small mammals in Central and South America. Causes outbreaks of disease in horses, donkeys and humans in endemic regions, occasionally spreading to southern USA
Western equine encephalitis virus	Mosquito (*Culex tarsalis* and other *Culex* species, *Aedes* species)	Infection of passerine birds widespread in the Americas. Causes mild disease in horses and humans
Getah virus	Mosquito	Causes sporadic disease in horses in south-east Asia and Australia characterized by fever, urticaria and oedema of the limbs. Subclinical infection occurs in pigs

64 *Flaviviridae*



The family name of the *Flaviviridae* (Latin *flavus*, yellow) is derived from yellow fever, a disease of humans caused by a flavivirus, with jaundice as a major clinical feature. Members of the family are 40 to 60 nm in diameter with icosahedral capsids and tightly adherent envelopes which contain either two or three virus-encoded proteins, depending on the genus. The genome is composed of positive-sense single-stranded RNA. Replication of virus occurs in the cytoplasm with maturation in cytoplasmic vesicles and release by exocytosis. The mature virions are generally labile, being sensitive to heat, detergents and organic solvents. The family comprises three genera. Two genera, *Flavivirus* and *Pestivirus* contain viruses of veterinary importance. The genus *Flavivirus* contains approximately 70 members assigned to several serologically defined groups. Most members of the genus are arboviruses, which require either mosquitoes or ticks as vectors. Viruses in the genus agglutinate goose red cells. The genus *Pestivirus* contains viruses of veterinary importance namely bovine viral diarrhoea virus, border disease virus and classical swine fever virus.

Clinical infections

In the genera *Flavivirus* and *Pestivirus* there are several viruses of particular veterinary importance (Table 64.1). Three members of the genus *Flavivirus*, louping ill virus, Japanese encephalitis virus and Wesselsbron virus, cause disease in domestic animals. In addition, infection with West Nile virus, an important human pathogen, causes fatal disease in horses. Other members of the genus which are important human pathogens include yellow fever virus, dengue virus, Japanese encephalitis virus, tick-borne encephalitis virus and St. Louis encephalitis virus. The sole member of the *Hepacivirus* genus, hepatitis C virus, causes hepatitis in humans.

The four recognised members of the *Pestivirus* genus which infect domestic species are closely related antigenically. Six distinct genotypes have recently been defined within the genus: classical swine fever virus, border disease virus, classical bovine viral diarrhoea virus (isolates predominantly from cattle), atypical bovine viral diarrhoea virus (isolates from cattle, sheep and pigs), deer pestivirus and giraffe pestivirus. Pestivirus infections may be inapparent, acute or persistent and are economically important worldwide.

Bovine viral diarrhoea and mucosal disease

Infection with bovine viral diarrhoea virus (BVDV) is common in cattle populations throughout the world. The virus can cause both acute disease, bovine viral diarrhoea (BVD), and a protracted form of illness, mucosal disease, arising from persistent infection. Using cell culture, cytopathic and non-cytopathic biotypes are recognized. The biotype most often isolated from cattle populations is non-cytopathic. Cytopathic isolates can arise from non-cytopathic BVDV as a result of recombination events, including incorporation of host RNA and duplication of viral RNA sequences. Two genotypes now considered separate species, BVDV 1 (classical BVDV isolates) and BVDV 2 (atypical BVDV isolates), are recognized on the basis of differences in the 5' untranslated region of the viral genome. Both genotypes contain cytopathic and non-cytopathic isolates, and produce similar clinical syndromes in cattle. However, only type 2 isolates have been associated with

Table 64.1 Viruses of veterinary importance in the genera *Flavivirus* and *Pestivirus*.

Genus	Virus	Hosts	Comments
Flavivirus	Louping ill virus	Sheep, cattle, horses, red grouse and humans	Present in defined regions of Europe. Transmitted by the tick *Ixodes ricinus;* produces encephalitis in sheep and other species
	Japanese encephalitis virus	Waterfowl, pigs, horses and humans	Widely distributed in Asia. Transmitted by mosquitoes. Waterfowl are reservoir hosts. Infection in pigs results in abortion and neonatal mortality
	Wesselsbron virus	Sheep	Occurs in parts of sub-Saharan Africa. Transmitted by mosquitoes. Produces generalized infection, hepatitis and abortion
	Israel turkey meningo-encephalomyelitis virus	Turkeys	Reported in Israel and South Africa. Transmitted by mosquitoes. Progressive paresis and paralysis
	West Nile virus	Birds, humans, horses	Birds are the natural hosts. Transmitted by mosquitoes. Serious nervous disease reported sporadically in humans and horses
Pestivirus	Bovine viral diarrhoea virus types 1 and 2	Cattle (sheep, pigs)	Occurs worldwide. Causes inapparent infection, bovine viral diarrhoea and mucosal disease. Congenital infection may result in abortion, congenital defects and persistent infection due to immunotolerance
	Border disease virus	Sheep	Occurs worldwide. Infection of pregnant ewes may result in abortion and congenital abnormalities
	Classical swine fever (hog cholera) virus	Pigs	Highly contagious, economically important disease with high mortality. Generalized infection with nervous signs and abortion; congenital tremors in piglets

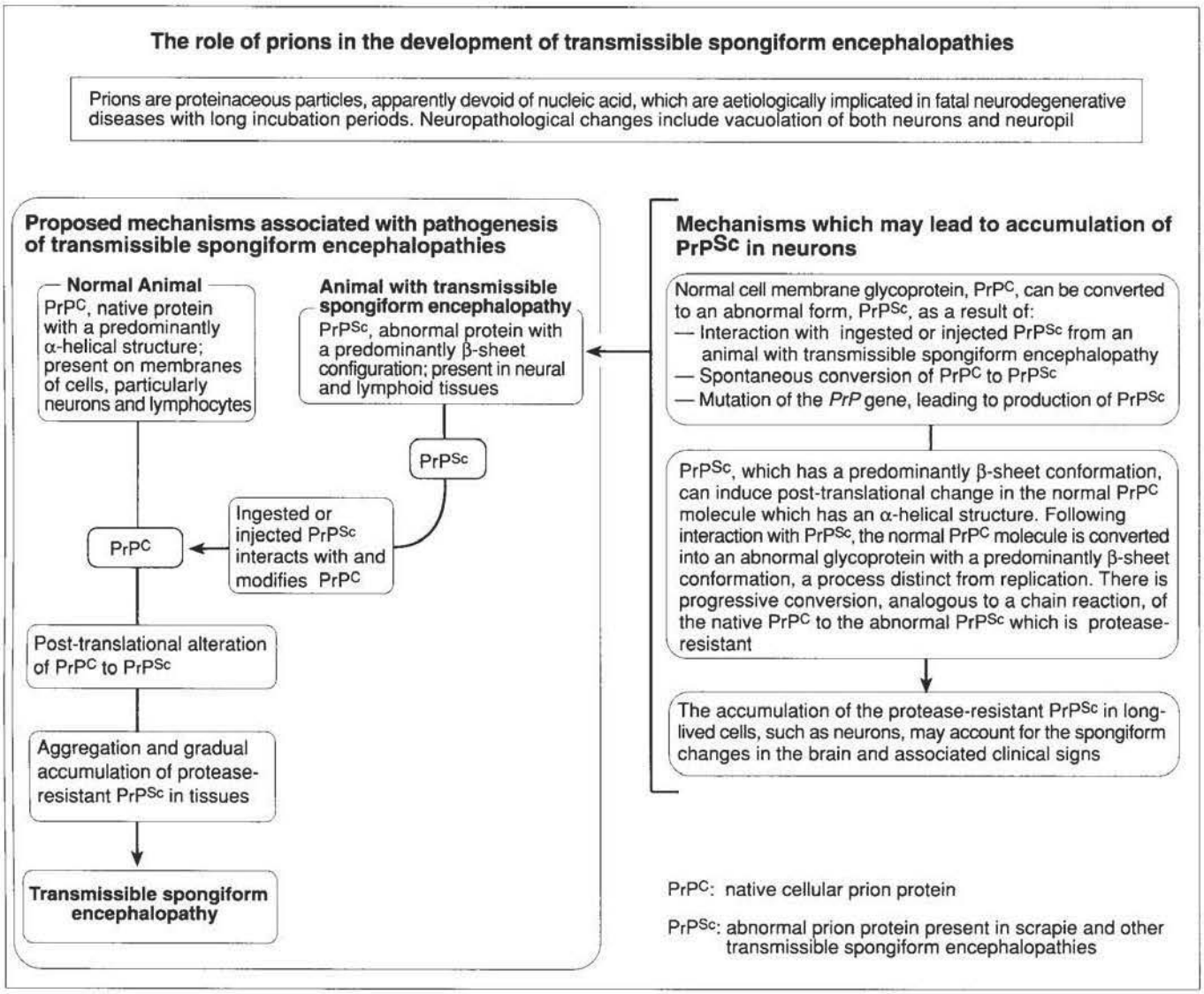
thrombocytopenia and a haemorrhagic syndrome, first described in North America. Isolates of BVDV used in vaccines and for diagnostic tests generally belong to the type 1 genotype.

Persistently-infected animals, which shed virus in secretions and excretions, are particularly important sources of infection. Persistent infection develops when infection of the foetus with a non-cytopathic strain occurs before day 120 of gestation. About 1% of animals in an infected population are persistently infected and viraemic. The presence of cattle with persistent infection in a herd results in constant exposure of the other cattle to virus, producing a high level of herd immunity. The outcome of transplacental spread depends on the age of the foetus at the time of infection. During the first 30 days of gestation, infection may result in embryonic death with return of the dam to oestrus. The effects of foetal infection between 30 and 90 days of gestation include abortion, mummification and congenital abnormalities of the CNS, often cerebellar hypoplasia. Foetuses which become infected after day 120 of gestation can mount an active immune response and are usually normal at birth. If virus invades the foetus before the development of immune competence, immunotolerance to the agent develops, with persistent infection for the lifetime of the animal. The virus involved in this persistent infection is non-cytopathic. Later, usually between six months and two years of age, a cytopathic biotype emerges as a consequence of mutation of the non-cytopathic virus or of recombination with nucleic acid of the host cell or other non-cytopathic biotypes. These events may lead to the development of mucosal disease in some animals.

Most BVDV infections are subclinical. Outbreaks of BVD are usually associated with high morbidity and low mortality. When present, clinical signs include inappetence, depression, fever and diarrhoea. Although a significant proportion of persistently-infected animals are clinically normal, some are born undersized and demonstrate retarded growth rate and poor viability. Mucosal disease is usually sporadic in occurrence. Clinical signs include depression, fever, profuse watery diarrhoea, nasal discharge, salivation and lameness. Ulcerative lesions are present in the mouth and interdigital clefts. Case fatality rate is 100%.

A tentative diagnosis may be possible on the basis of clinical signs and pathological findings. Laboratory confirmation requires demonstration of antibody, viral antigen or viral RNA. Seroconversion and the presence of viraemic animals are necessary for confirmation of established infection in a herd. Most losses arising from BVDV infections in herds result from the effects of prenatal infections and mucosal disease. Control strategies are directed at preventing infections which can lead to the birth of persistently-infected animals. Killed, attenuated live and temperature-sensitive mutant virus vaccines have been developed. The elimination of BVDV from a herd requires the identification and removal of persistently-infected animals. Systematic testing of bulk milk or pooled blood samples for antibodies is important in national eradication programmes.

65 Prions: unconventional infectious agents



At present, conventional infectious agents have not been implicated aetiologically in the transmissible spongiform encephalopathies (TSEs), a unique group of neurodegenerative diseases. It has been proposed that these diseases are caused by unconventional infectious agents termed prions. These infectious agents are 'unconventional' because they appear to be devoid of nucleic acid, unlike viruses and other microbial agents. In addition, they are non-immunogenic and are extremely resistant to inactivation by heating, exposure to chemicals and irradiation. The 'prion theory' proposes that they are derived from a native glycoprotein PrP^C (cellular prion protein), associated with the plasma membrane of many cell types. Following exposure to abnormal prion protein (PrP^{Sc}, scrapie prion protein), PrP^C is altered post-translationally to a structure similar to that of the PrP^{Sc}. As more PrP^C is converted to PrP^{Sc}, this protease-resistant molecule gradually accumulates, especially in the long-lived cells of the CNS. The formation of PrP^{Sc} from PrP^C in TSEs may be initiated following exposure to an external source of PrP^{Sc}, usually by ingestion. Rarely, random spontaneous conversion of native PrP^C to PrP^{Sc} may initiate the process in an individual. A third mechanism which predisposes to configurational change in PrP^C relates to mutation in the *PrP* gene, as occurs in the Gerstmann-Sträussler-Scheinker syndrome in humans.

The *PrP* gene of an infected animal determines the primary amino acid sequence of the prion protein in that animal. The resistance of some species to infection by prions derived from another species is termed the 'species barrier'. This barrier is attributed to differences between the amino acid sequences of the prion proteins in the two species. On initial transfer of PrP^{Sc}

Table 65.1 Transmissible spongiform encephalopathies of animals.

Disease	Comments
Scrapie	Recognized in sheep in parts of Europe for 300 years; apart from Australia and New Zealand, now occurs worldwide. Occurs also in goats
Bovine spongiform encephalopathy	First reported in England in 1986; developed into a major epidemic over a ten-year period. Prevalence declined with the implementation of effective control measures. Occurs at low frequency in many other European countries
Feline spongiform encephalopathy	First recorded during the bovine spongiform encephalopathy epidemic in the early 1990s. Most cases reported in the UK
Transmissible mink encephalopathy	First recognized in caged mink in Wisconsin in 1947; attributed to the feeding of scrapie-infected sheep meat
Spongiform encephalopathy in captive ruminants	First recorded during the bovine spongiform encephalopathy epidemic in 1986. Reported in greater kudu, nyala, oryx and some other captive ruminants in zoological collections
Chronic wasting disease	First recognized in captive mule deer in Colorado in 1980. Occurs in deer and elk populations in the wild in North America

between species, the incubation period tends to be relatively long. Subsequent transfer between members of the recipient species leads to shorter incubation periods. The presence of a 'species barrier' may explain the resistance of humans to infection with PrP^{Sc} derived from sheep with scrapie.

Diseases attributed to prions occur sporadically and are significantly influenced by the genome of the affected animal. These slowly progressive neurodegenerative diseases, which are characterized by long incubation periods and spongiform changes in the brain, have been described in many animal species and in humans. Transmissible spongiform encephalopathies have been recognized in both ruminants and carnivores (Table 65.1). In scrapie, there is convincing evidence for the importance of the genetic constitution of certain breeds of sheep in determining susceptibility to the disease.

Scrapie

This insidious, fatal, neurological disease of adult sheep and goats occurs worldwide, except in Australia and New Zealand. The mode of transmission of scrapie is not clearly understood. The disease has a long incubation period. Neurological signs develop predominantly in sheep of breeding age with a peak incidence between three and four years of age. Initially, affected animals may present with restlessness or nervousness, particularly after sudden noise or movement. Pruritis may result in loss of wool. Progression of the disease leads to emaciation. Death usually occurs within six months from the onset of clinical signs. Clinical signs and histopathological examination of the CNS form the basis for diagnosis. Characteristic microscopic changes include neuronal vacuolation and degeneration, vacuolar change in the neuropil and astrocytosis, particularly in the medulla. No obvious inflammatory response is evident. Confirmatory methods include immunohistochemical staining for PrP^{Sc}, immunoblotting to detect proteinase-K-resistant PrP^{Sc} and electron microscopy to detect scrapie-associated fibrils in detergent-treated extracts of brain.

In the European Union, scrapie has been designated a notifiable disease. Slaughter policies have been enforced with different degrees of success in several countries. In Australia and New Zealand an eradication policy, implemented soon after the introduction of the disease, was successful. Eradication was abandoned in the United States because of the cost and difficulties involved in its implementation. Breeding scrapie-resistant sheep may be a realistic method for reducing the frequency of the disease.

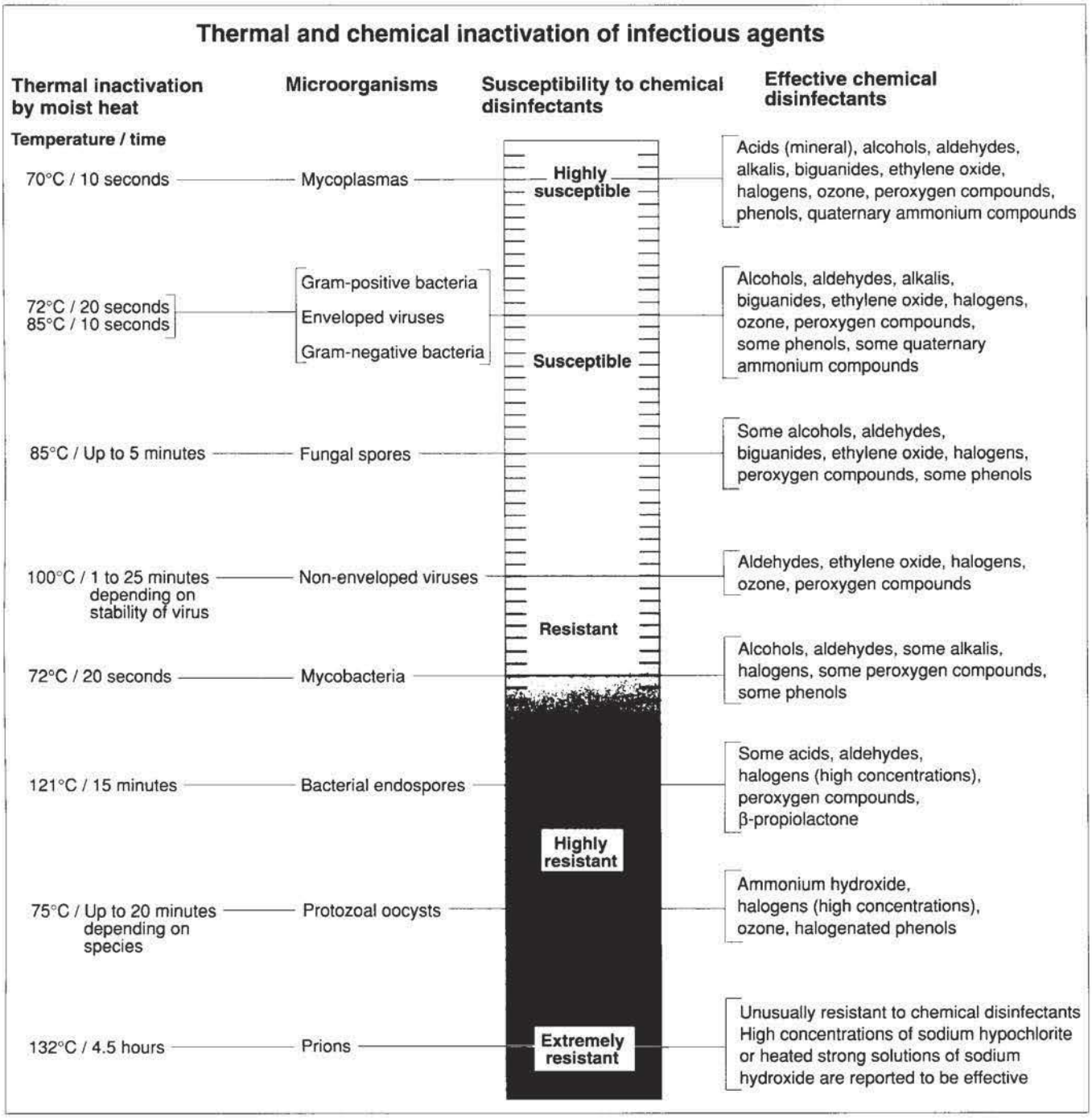
Bovine spongiform encephalopathy

This condition is a progressive, neurodegenerative disease of adult cattle, first recognized in England in 1986. More than 170,000 cases of the disease were subsequently confirmed and an estimated one million animals were infected. The disease has been reported in several countries in animals imported from Great Britain. In addition, indigenous cattle in a number of European countries, including Switzerland, Ireland, France and Portugal, have developed the disease.

The prion strain causing bovine spongiform encephalopathy (BSE) is not considered to be species-specific. In 1996, a novel form of human prion disease, termed variant Creutzfeldt-Jakob disease (vCJD) was recognized in Great Britain. Molecular strain-typing studies and experimental transmission in transgenic and conventional mice indicated that vCJD and BSE are caused by indistinguishable prion strains. The BSE epidemic in Great Britain was attributed to contaminated meat-and-bone meal (MBM) prepared from slaughterhouse offal and fed as a protein dietary supplement to cattle. It is postulated that the scrapie agent crossed the species barrier into cattle in the early 1980s, following changes in the rendering process which allowed survival of increased amounts of scrapie PrP (PrP^{Sc}) in MBM. As a result of the banning of ruminant-derived MBM in 1988, there was a marked decline in the prevalence of BSE in Great Britain after 1993. Horizontal transmission of BSE does not appear to occur. The mean incubation period is about five years. Neurological signs, which are highly variable, include changes in behaviour and deficits in posture and movement. Ataxia, hypermetria and a tendency to fall become increasingly evident in the later stages of the disease. The clinical course may extend over many days or months.

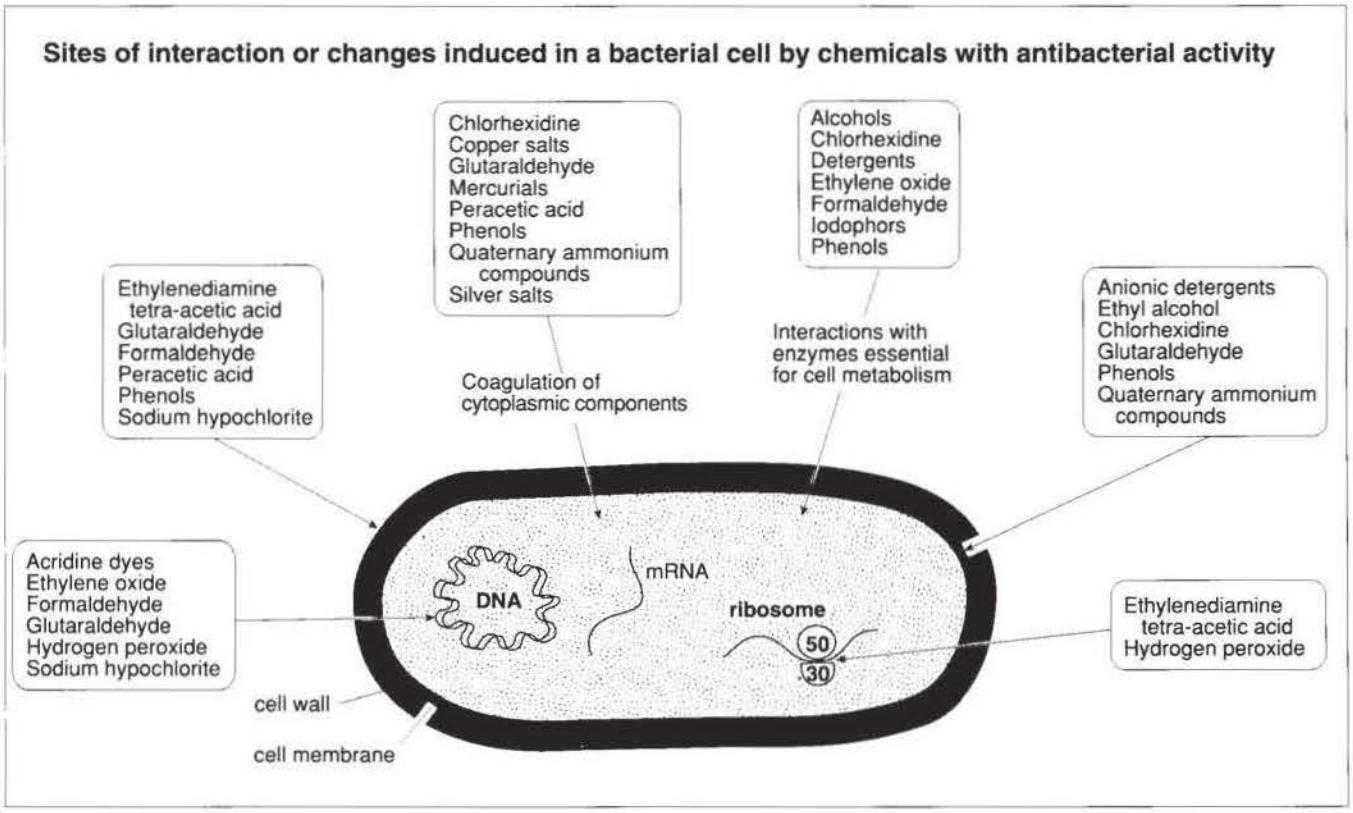
Bovine spongiform encephalopathy can be confirmed by histopathological examination of brain tissue and specific immunological confirmatory methods. Bovine spongiform encephalopathy is a notifiable disease in countries of the European Union. Control is based on slaughter of affected animals and exclusion of ruminant-derived protein from ruminant rations.

66 Disinfection



Infectious agents shed in excretions or secretions of animals, or present in products of animal origin, may remain viable for long periods in the environment. Buildings, transport vehicles, soil, pasture, water and fomites may become contaminated by faeces or urine containing bacterial, viral or protozoal pathogens. Fungal pathogens such as dermatophytes may contaminate surfaces and grooming equipment. Respiratory secretions of sick animals may contain viral or bacterial pathogens and, following abortion, high numbers of infectious agents such as *Brucella abortus* may be present in foetal fluids.

During an outbreak of infectious disease, isolation of infected and in-contact animals is used to limit spread. If the disease is exotic or subject to a national eradication programme, laboratory testing of clinically affected animals is followed by



slaughter of infected and in-contact animals. For endemic infectious diseases, vaccination, disinfection, chemotherapy and chemoprophylaxis are employed selectively depending on the aetiological agents and the methods appropriate for their control. Disinfection is an essential part of disease control programmes both for endemic and exotic diseases. It is also used to minimise the risk of disease transmission from animals to humans not only during the production phases but also at the processing stages in meat plants and dairies.

Disinfection implies the use of physical or chemical methods for the destruction of microorganisms, especially potential pathogens, on surfaces of inanimate objects or in the environment. Infectious agents vary widely in their susceptibility to thermal inactivation. Although both moist and dry heat can be used for inactivating microorganisms, moist heat is more effective and requires less time to achieve inactivation than dry heat. At temperatures above 80°C, most vegetative bacteria are killed within seconds. Bacterial endospores are remarkably thermostable and moist heat at 121°C for at least 15 minutes is required for their destruction. Many enveloped viruses are labile at temperatures close to 70°C. Non-enveloped viruses such as foot-and-mouth disease virus are thermostable and temperatures close to 100°C for more than 20 minutes may be required to inactivate such resistant viruses. The prions which cause transmissible spongiform encephalopathies are extremely resistant to thermal inactivation. Dry heat at 160°C does not inactivate these agents. Autoclaving at 132°C for 4.5 hours is required for their inactivation.

Infectious agents vary widely in their susceptibility to chemical disinfectants. Most vegetative bacteria and enveloped viruses are readily inactivated by disinfectants; fungal spores and non-enveloped viruses are more resistant to chemical inactivation. Mycobacteria and bacterial endospores are resistant to many commonly used disinfectants. Prions are extremely resistant to chemical inactivation. High concentrations of sodium hypochlorite or heated strong solutions of sodium hydroxide are reported to inactivate these resistant infectious agents.

Chemical compounds with antibacterial activity may react with the cell wall, cell membrane, nucleic acid and other cytoplasmic constituents. Virucidal disinfectants may react with nucleic acids, structural or functional proteins, glycoproteins, or in the case of enveloped viruses, with the lipid envelope.

For the success of a disinfection programme, thorough cleaning should precede the application of disinfectant. The disinfectant selected should be active against the infectious agents present and it should be diluted to yield the correct concentration. Most disinfectants require several hours to inactivate infectious agents on surfaces; the presence of organic matter such as faeces, exudates or body fluids interferes with the antimicrobial activity of disinfectants and slows their action. Failure to inactivate infectious agents present in buildings, on equipment or in transport vehicles may be due to the selection of an ineffective disinfectant, careless use of a potentially effective disinfectant or environmental factors. As no residual antimicrobial activity persists after disinfection, infectious agents may be reintroduced by infected animals, fomites, food, personnel or rodents.

Index

All numbers refer to page numbers.